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Phylogeny of Hinterhubera, Novenia and related genera based on the nuclear ribosomal (nr) DNA sequence data (Asteraceae: Astereae)

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PHYLOGENY OF *HINTERHUBERA*, *NOVENIA* AND RELATED GENERA BASED ON THE NUCLEAR RIBOSOMAL (nr) DNA SEQUENCE DATA (ASTERACEAE: ASTEREAE)

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"Treat the earth well: it was not given to you by your parents, it was loaned to you by your children. We do not inherit the Earth from our Ancestors, we borrow it from our Children."

Ancient Indian Proverb

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ABSTRACT

Analyses of ITS, ETS and matK sequences for genera of the subtribe Hinterhuberinae, including representatives of 17 subtribes from the tribe Astereae, rooted on Anthemideae, Calenduleae and Inuleae, provide no support for the monophyly of the Hinterhuberinae. *Chiliotrichum* and *Celmisia* groups, *Olearia* in part, *Novenia*, *Oritrophium* and *Pteronia* are in the basal group that also includes subtribe Felicinae. *Madagaster, Remya*, *Olearia* in part, *Achnophora* and *Hinterhubera* group lie in the Southern Hemisphere grade, with Asterinae, Bellidinae, Brachycominae, Grangeinae, Podocominae, Lagenophorinae and Baccharidinae. Monophyly of Asterinae, Grangeinae, Podocominae and Lagenophorinae is not supported. Instead South American representatives of Lagenophorinae, Grangeinae and Podocominae group with Baccharidinae and *Hinterhubera* group in the South American clade that is nested in the Southern Hemisphere grade. They are sister to the North American subtribes. *Remya, Achnophora, Olearia* in part and Australasian, European and African representatives of Asterinae, Brachycominae, Grangeinae, Podocominae and Lagenophorinae are grouped in a polytomy that is basal to the South American clade. *Laennecia* (Podocominae) is sister to *Westoniella* (Hinterhuberinae), and *Myriactis panamensis* (Lagenophorinae) and *Plagiocheilus* (Grangeinae) group with *Archibaccharis* (Baccharidinae). The *Celmisia* group is monophyletic. The radiate *Diplostephium* and *Guynesomia* do not group with other radiate genera from the *Chiliotrichum* group, but are in the Southern Hemisphere grade and close to the disciform *Hinterhubera* group. Transfer of *Blakiella* from Podocominae to Hinterhuberinae is supported by the three datasets. *Madagaster* is isolated from the other Hinterhuberinae in having basal position in the Southern Hemisphere grade. Evolution of dioecy in Baccharidinae and *Aztecaster* (Hinterhuberinae) evolved independently. Woody

habit, epaleate receptacle, bisexual disk florets and ligulate peripheral florets are ancestral states in the tribe Astereae. Staminate disk florets and paleate receptacles are derived characters that evolved in several lineages independently. South America, southern Africa or New Zealand are proposed centers of origin for Astereae and for the *Chiliotrichum* and *Celmisia* groups. The *Hinterhubera* group evolved from the South American autochthonous elements, probably from the lower altitude plants during the uplift of the Andes and the development of páramo habitat.

CHAPTER 1 INTRODUCTION AND LITERATURE REVIEW

Family Asteraceae is the largest family of vascular plants with more than 1500 genera and ca. 23,000 species arranged in 11 subfamilies and 35 tribes (Bremer 1994, Panero and Funk 2002). Tribal classification of the family dates back to the works of Cassini (1816, 1821), Lessing (1832) and De Candolle (1836). Bentham (1873) and Hoffman (1890) described 13 tribes in the family Asteraceae, and their systems of classification, although modified, are still widely accepted and followed. Main changes in the classification of the tribes occurred in the last forty years (Nordenstam 1977, Anderberg 1989, 1991a, 1991b, 1991c, Bremer and Jansen 1992, Robinson and Funk 1987, Karis et al. 1992, Gadek et al. 1989, Bremer and Humphries 1993). Molecular studies based on comparative DNA sequence data of chloroplast genes and nuclear ribosomal spacers have provided new insights into the evolution and radiation of the family, supporting the segregation of new tribes, subtribes and subfamilies (Jansen and Palmer 1987, Jansen and Kim 1994, Kim and Jansen 1995, Panero and Funk 2002, Kim et al. 2005).

The tribe Astereae includes approximately 3000 species in nearly 200 genera. The tribe as recently treated contains 18 subtribes (Nesom and Robinson 2006) with worldwide distribution and a center of greatest diversity in North and South America. Astereae are a well defined, monophyletic tribe (Grau 1977). It was divided into six subtribes (Bentham 1873), a classification which Hoffmann (1890) adopted almost unchanged. Most of the genera were grouped in two subtribes based on the color of ray and disk florets: genera with yellow ray and disk florets were in the subtribe Homochromeae Benth. (=Solidagininae Hoffm.) and genera with yellow disk and white, purple or blue ray florets in the subtribe Heterochromeae

Benth. (=Asterinae Dumort). Grau (1977) found this classification unnatural, indicating many exceptions to the strictly homochromous and heterochromous conditions within genera, and thus obscuring the distinction between Heterochromeae and Homochromeae. He accepted only the subtribes Grangeinae and Baccharedinae as natural groups, while the rest of the genera of Astereae he grouped according to their geographical distribution.

Unusual, deeply 5-lobed actinomorphic to zygomorphic tubular ray florets of *Hinterhubera* were unique in the tribe Astereae. Cuatrecasas (1969) considered that such florets in combination with its shrubby habit and coriaceous leaves were sufficiently distinct for establishment of a new subtribe, Hinterhuberinae. Later he (1986) expanded the subtribe to include *Westoniella, Blakiella* and *Flosmutisia*. Hinterhuberinae were defined as shrubby plants with zygomorphic to actinomorphic, tubular female florets, sterile disk florets, densely glandular and hispid cypselae and ovaries, and the cypselae with "twin-type" trichomes.

Cladistic analysis of the tribe Astereae based on morphological data (Zhang and Bremer 1993, Bremer 1994) resolved only three subtribes in the tribe Astereae, Grangeinae, Solidagininae and Asterinae *sensu lato*, coinciding with the Bentham's (1873) and Hoffmann's (1890) homochromous/ heterochromous division of the tribe. The Hinterhuberinae (*sensu* Cuatrecasas 1986), Bellidinae, Conyzinae and Baccharidinae, were nested within their Asterinae (Heterochromeae). Being distributed in the Southern Hemisphere the genera from the subtribe Hinterhuberinae represent a group separate from the primarily Northern Hemisphere Asterinae (Nesom 1993a). Hinterhuberinae were redefined and expanded to include mostly shrubby genera, with *Oritrophium* being the only herbaceous and thus the most derived member of the subtribe (Nesom 1993a). Initially, Nesom (1993a) excluded *Westoniella*, *Flosmutisia* and *Blakiella* from this redefined subtribe, but later he

concluded that the two former genera plus *Floscaldasia* are among the most specialized elements in Hinterhuberinae (Nesom 1993b, 1994a). The genus *Blakiella* he placed within the subtribe Podocominae (Nesom 1994a).

In the treatment of the tribe Astereae based on morphological characteristics Nesom (1994a) recognized 29 genera within the subtribe Hinterhuberinae that he classified in seven informal groups (tab. 1.1). Since his synoptical treatment, several studies on the taxonomy of certain genera have been completed. Phylogenetic analyses of nrDNA sequence data (Noyes and Rieseberg 1999, Roberts and Urbatsch 2003, Urbatsch et al. 2003) suggested exclusion of *Ericameria* Nutt. from Hinterhuberinae and its possible transfer to Solidagininae. Two monotypic genera, *Aylacophora* and *Paleaepappus* from Patagonia, based on receptacular and pappus morphology were reinstated from *Nardophyllum* (Bonifancino and Sancho 2001). *Nardophyllum scoparium* Phil., an anomalous species in the genus (Nesom 1993d), was the basis for the new genus, *Guynesomia* (Bonifancino and Sancho 2004). And finally, *Blakiella* was transferred from Podocominae back to Hinterhuberinae (Nesom and Robinson 2006). Currently the subtribe includes 32 genera primarily distributed in Southern Hemisphere: South and Central America, southern Africa, Madagascar, Australia, New Zealand and its sub-Antarctic islands, with two genera found in Mexico (tab. 1.1).

ECOLOGICAL ASPECTS OF HINTERHUBERINAE

Hinterhubera, *Novenia* and related genera are mainly microphyllous, prostrate dwarf shrubs that grow in the tropical alpine vegetation zone: the páramos of the northern Andes, the punas of the Andean Altiplano, and steppes in Patagonia. Genera centered around *Celmisia* and *Olearia* are distributed mainly in the alpine vegetation belt of Australasia, with a

Table 1.1 List of genera from the subtribe Hinterhuberinae according to Nesom and Robinson (2006). Classification into groups follows Nesom (1994a). Three genera are unplaced, *Blakiella* was recently transferred from Podocominae to Hinterhuberinae, *Guynesomia* is a newly described genus with possible affinity to the *Hinterhubera* group, and *Printzia* was transferred from Inuleae to Hinterhuberinae.

GROUP	GENUS [number of species]	DISTRIBUTION
Celmisia	***Achnophora*** F. Muell. [1]	South Australia
	Celmisia Cass. [ca. 60]	New Zealand, South Australia, Tasmania
	Damnamenia Given [1]	Auckland and Campbell Islands
	Olearia Moench in part	New Zealand
	Pachystegia Cheeseman [1]	New Zealand
	Pacifigeron Nesom [1]	French Oceania
	Pleurophyllum Hook. f. [3]	subantarctic islands of New Zealand
Chiliotrichum	***Aylacophora*** Cabrera [1]	Patagonia
	Chiliophyllum Phil. [3]	Argentina, Chile
	Chiliotrichiopsis Cabrera [4]	Argentina, Chile, Peru
	Chiliotrichum Cass. [2]	Argentina, Chile
	Diplostephium H.B. & K. [ca. 80]	Chile, Peru, Bolivia, Ecuador, Colombia, Panama, Costa Rica
	Lepidophyllum Cass. [1]	Patagonia
	Llerasia Triana [14]	Bolivia, Peru, Ecuador, Colombia
	Nardophyllum Hook & Arn. [5]	Argentina, Chile, Bolivia
	Oritrophium (H.B. & K.) Cuatr. [20]	Peru, Bolivia, Ecuador, Colombia, Venezuela, Mexico
	Paleaepappus Cabrera [1]	Patagonia
	Pteronia L. [ca.80]	South Africa, Zimbabwe
Hinterhubera	***Aztecaster*** Nesom[2]	Mexico
	Floscaldasia Cuatr. [2]	Ecuador, Colombia
	Flosmutisia Cuatr. [1]	Colombia
	Hinterhubera Sch. Bip. [8]	N Chile, Colombia, Venezuela
	Laestadia Kunth [6]	Peru, Bolivia, Ecuador, Colombia, Venezuela, Hispaniola, Costa Rica
	Parastrephia Nutt. [5]	Peru, Chile, Argentina
	Westoniella Cuatr. [6]	Costa Rica, Panama
Madagaster	***Madagaster*** Nesom [5]	Madagascar
	Mairia Nees [14]	South Africa

table 1.1 continued

	Rochonia DC. [4]	Madagascar
Novenia	***Novenia*** Freire [1]	Peru, Bolivia, Argentina
Olearia	***Olearia*** Moench in part	Australia
Remya	***Remya*** Hillebrand [3]	Hawaii
Unplaced	***Blakiella*** Cuatr. [1]	Colombia, Venezuela
	Guynesomia Bonifancino & Sancho [1]	Chile
	Printzia Cass. [6]	South Africa

few species within the lowland, coastal zone. Páramo is an open type of vegetation above the upper forest line in the tropical Andes of Venezuela, Colombia, Ecuador and northern Peru with outliers in Costa Rica, Panama and in the humid eastern Andean slopes in Peru and Bolivia. Its climate is humid with precipitation almost evenly distributed throughout the year, except for a short dry period generally in December and January. Large diurnal variations in the temperature are typical for this ecosystem. The vegetation is dominated by grasses, principally *Calamagrostis* Adans., and Asteraceae. Puna vegetation occurs in the Andean Altiplano highlands of central Peru, Bolivia and Argentina, and in contrast to páramo is typically xeric with lower humidity and shorter wet season. Precipitation is absent for six months or more per year. Its sparse vegetation changes from open tussock-grass shrubland in the driest western part to a more meadow-like grass-scrub formation in the wetter eastern section. The climate of Patagonia is typically classified as arid temperate cold climate. The vegetation includes steppe dominated by grasses, or a steppe with shrubs and trees mixed with grasses. The humidity level is one of the factors that determines the dominance of the life forms in different zones. Thus tall, shrubby forms are found to be dominant in dry environments, and herbaceous perennials and mat forming subshrubs in more humid environments of the Andean alpine vegetation belts (Arroyo et al. 1993). Similarly

Parastrephia, *Lepidophyllum*, *Chiliotrichum* etc. are better adapted to the dry environments of the puna region and Patagonia, while perennial herbaceous genera such as *Oritrophium* and *Flosmutisia*, and mat forming subshrubs like *Floscaldasia, Westoniella,* and *Hinterhubera* prefer the wetter páramo belt.

Most high elevation tropical plants have a characteristic physiognomy that is expressed in the form of rosette and cushion plants, and microphyllous and prostrate dwarf shrubs. These growth forms are repeated in geographically disjunct, high elevation regions of the world such as the páramos and punas of South America and the high mountains of eastern Africa (Cuatrecasas 1968). Having evolved independently in several different lineages these similar growth forms are examples of convergent evolution in response to the unique high elevation environments. Investigators have long recognized that morphological convergence is frequently present in plants adapted to similar types of habitat (Troll 1948, Hedberg 1964, 1973, Small 1973). Such convergence in morphology often confounds their taxonomy as distantly related taxa are frequently treated as closely related. Numerous such cases occur in the tribe Astereae (Asteraceae). For example, based on the similarity of cypselae morphology *Kalimeris* Cass. and *Boltonia* L'Hér. were considered monophyletic (Gu and Hoch 1997); *Oritrophium* was once a section of *Celmisia* (Solbrig 1960); dioecious *Aztecaster* was placed in *Baccharis* L. (Rzedowski 1972); similarity in ray florets and pappus morphology placed Madagascan and Cape Verde species of *Conyza* L. within *Nidorella* Cass. (Wild 1969); and similar overall morphology resulted in *Batopilasia* Nesom & Noyes and *Chloracantha* Nesom, Suh, Morgan, Sundb. & Simpson being treated as *Erigeron* L. (Sundberg and Nesom 1990, Nesom 2000, Nesom and Noyes 2000), just to mention few examples. *Hinterhubera, Novenia* and related genera are distributed in the tropical alpine vegetation zones. Their treatment

within the subtribe Hinterhuberinae is based on the presence of similar coriaceous leaves, shrubby habit, dense tomentum, and pubescent, glandular, multinerved cypselae. However, molecular analyses of the ITS sequence data (Noyes and Rieseberg 1999) that included several genera from Hinterhuberinae, indicated the possible polyphyletic nature of the subtribe suggesting that these characters may not be synapomorphic, but the result of convergent evolution and adaptation to the high altitude ecosystems. The present study investigates these possibilities using sequence data of the nrDNA as an independent test of phylogeny.

SYSTEMATICS AND TAXONOMY OF HINTERHUBERINAE

The *Chiliotrichum* group (*sensu* Nesom 1994a) is the largest and includes genera distributed in South America. Species in this group are shrubby, with coriaceous leaves, radiate capitula (*Nardophyllum* has discoid capitula), paleate receptacles, and hermaphroditic disk florets (*Lepidophyllum* has staminate disk florets and epaleate receptacle). Members of the group have various taxonomic treatments (Bentham 1873, Cabrera 1945, 1954, Nesom 1991, 1993a, Nesom et al. 2001, Zhang and Bremer 1993; Bremer 1994; Bonifancino and Sancho 2001, 2004). Because of the yellow flowers and somewhat "*Solidago*-like" style appendages that are present in *Aylacophora*, *Chiliophyllum*, *Chiliotrichum*, *Chiliotrichiopsis*, *Nardophyllum* and *Parastrephia*, Nesom (1991) speculated that this group belonged within subtribe Solidagininae, although the nature of the relationship to the rest of the Solidagininae was unclear. In the morphology-based cladogram of Zhang and Bremer (1993), the *Chiliotrichum* group represented by the genus *Chiliotrichum*, was well nested within their Asterinae subtribe. They expanded the group to include *Diplostephium* and *Lepidophyllum*, while two other genera, *Llerasia* and *Pteronia* were placed in their subtribe Solidagininae, as

part of the *Haplopappus* and *Engleria* groups, respectively (Zhang and Bremer 1993, Bremer 1994). Molecular analyses of ITS data (Noyes and Rieseberg 1999, Cross et al. 2002) that included five genera from Hinterhuberinae, *Diplostephium, Chiliotrichum, Olearia, Oritrophium* and *Pteronia*, suggested only a distant relationship of *Diplostephium* to the *Chiliotrichum* group. The latter four genera were in the basal group with Felicinae. *Diplostephium* was, however, well nested within the Southern Hemisphere grade thus being only distantly related to the remaining four genera.

The *Madagaster* group includes three genera, the woody yellow-rayed *Rochonia*, the white rayed *Madagaster,* both distributed in Madagascar, and the herbaceous southern African *Mairia* which has white or purple ray florets. The species of *Madagaster* were initially described within the genus *Aster* L. (Humbert 1960). Zhang and Bremer (1993) and Bremer (1994) maintained these white-rayed species in *Aster*. Nesom (1993c), however, did separate them into the genus *Madagaster* because of its distinctive features that include woody habit, pannose tomentum, coriaceous leaves and multinerved terete cypselae. He suggested *Madagaster*'s possible close affinities to *Rochonia* and rejected its hypothesized close relationship to *Olearia* or *Diplostephium* (Nesom 1993c). *Mairia* may be a herbaceous derivative of woody ancestors, such as *Rochonia, Madagaster* or some other members of the subtribe (Nesom 1994b).

The *Olearia* and *Celmisia* groups include genera distributed in Australasia. *Olearia* is the largest genus of Astereae in Australasia; its species exhibit variable morphologies in habit, capitulescence and tomentum, and unusually high level of polyploidy in many cases. Several authors suggested or provided evidence that *Olearia*, as presently delimited, is polyphyletic (Drury 1968; Given 1973; Given and Gray 1986; Nesom 1993c, 1994a, Cross et al 2002) with

some species more closely related to *Celmisia, Pleurophyllum,* or *Pachystegia* than to each other. *Celmisia* is an element of the alpine floras in Australia and New Zealand, and was hypothesized closely related to *Oritrophium* from South America (Solbrig 1960). The two differ in the sterility of disk florets and habit, features that Cuatrecasas (1961) considered significant in maintaining them as separate genera.

Hawaiian genus *Remya* is unusual in the subtribe because it is the only genus with two types of disk florets. In two of its three species, the outer series of disk florets are neuter or pistillate, and the inner series are staminate. Tomentum on the leaves, inflorescence type, shape of receptacle and morphology of the cypselae indicated close relationship to the New Zealand species of the genus *Olearia* (Wagner and Herbst 1987). Cross et al. (2002) in their ITS-based analysis did not confirm this hypothesis, suggesting instead that those characters were symplesiomorphic in the tribe or developed independently in several lineages within Astereae.

TAXONOMIC REVIEW OF THE *HINTERHUBERA* AND *NOVENIA* GROUPS

The informal *Hinterhubera* group (*sensu* Nesom 1994a) includes five genera distributed in South America, one genus in Central America and one genus in Mexico. The genus *Hinterhubera* was described by Schultz Bipontinus, but it was published for the first time by Weddell (1857). Weddell placed the genus in Baccharideae, but remarked that it could as well be in Astereae together with *Diplostephium*. Cuatrecasas (1969) recognized the unique morphology of its ray corollas and established the new subtribe Hinterhuberinae to accommodate the genus. *Flosmutisia*, a monotypic genus from Colombia, based on its flat cypselae, highly modified ray corollas, and disc flowers with sterile ovaries was considered

closely related to *Hinterhubera* (Nesom 1993b), and *Westoniella* and *Blakiella* (Cuatrecasas 1986).

The name of the genus *Parastrephia* is derived from the Greek word παραστρεφω = to invert, implying the inverted position of the florets in the capitulum, i.e. tubular male florets at the periphery and filliform female disk florets in the center of the capitulum (Nuttall 1841). This observation was based either on abnormal samples or it was an error in observation (Bentham 1873, Blake 1930). Blake studied the original material and identified it as *Lepidophyllum phylicaeforme* Hieron, thus placing *Parastrephia* in the synonymy of *Lepidophyllum*. Alternate leaves, and reduced ray florets in five *Lepidophyllum* species from the puna region in Bolivia and surrounding areas in Peru, Chile and Argentina were considered significantly different from the opposite leaved and radiate *L. cupressiforme* Cass. from Patagonia and therefore were transferred to *Parastrephia* (Cabrera 1954). At present, only three species are recognized within *Parastrephia* (Nesom 1993d).

The genus *Westoniella* was named in the honor of Dr. Arthur S. Weston who collected all the six species on the massifs of Chirripó and Buenavista, Costa Rica (Cuatrecasas 1977), and on the massif of Fábrega, Panama (Cuatrecasas 1982). One of the species, *Westoniella eriocephala* (Klatt) Cuatr., had previously been described in *Senecio* L. and later transferred to *Erigeron* (Cuatrecasas 1977). The reduced, tubular corollas of the ray florets were hypothesized to be primitive structures in the tribe Astereae, hence the genus was initially placed within the subtribe Asterinae (Cuatrecasas 1977). Later Cuatrecasas (1986) recognized a derived origin of such corollas and consequently transferred the genus to Hinterhuberinae.

Taxonomy of *Laestadia* has been controversial. The genus was regarded closely related to *Lagenophora* Cass., *Solenogyne* Cass., *Myriactis* Less., *Keysseria* Lauterbach

(Cabrera 1966). Because of the similarity in the structure of exothecial cells of the anthers, *Laestadia* was suggested to be related to *Plagiocheilus* Arn. ex DC., *Lagenophora* and *Floscaldasia*, and placed in the subtribe Grangeinae (Robinson and Bretell 1973). However, *Laestadia* was excluded from Grangeinae (Fayed 1979) and tentatively placed within Bellidinae (Cuatrecasas 1986). Its thick, entire, linear leaves and 6-10 nerved cypselae suggest its relationship to specialized members of Hinterhuberinae, while its similarities to Grangeinae were attributed to convergence (Nesom 1993b, 1994a). *Floscaldasia* was often misidentified as *Laestadia* because of their remarkably similar creeping habit and reduced leaves (Nesom 1993b, Sklenář and Robinson 2000). The former differs from *Laestadia* in the presence of pappus bristles. *Floscaldasia* has variously been considered a member of subtribes Asterinae (Cuatrecasas 1969), Grangeinae (Robinson and Brettell 1973) or Bellidinae (Cuatrecasas 1986). Bremer (1994) accepted Robinson's and Brettell's hypothesis of the close relationship of *Laestadia* and *Floscaldasia*, but kept them in his *Lagenophora* group within the tribe Astereae. At present, the genus is regarded among the most specialized elements of the subtribe together with *Laestadia*, *Westoniella* and *Flosmutisia* (Nesom 1994a).

The monotypic *Novenia* grows in high elevations of the Peruvian Andes. Initially it was treated in the subtribe Gnaphaliinae of Inuleae (Freire 1986; Freire and Hellwig 1990), it was related to the genera of the Inuleae *sensu stricto* (Anderberg 1989), or listed within genera with unknown position in the subfamily Asteroideae (Anderberg 1991a, Bremer 1994). The highly reduced and specialized vegetative, phyllary and floral morphology, the morphology of anthers, cypselae and pappus, as well as the chromosome number place it in Astereae. Its rigid leaves, auxiliary tufts of tomentum, reduced pistillate corollas, functionally

staminate disk florets, multinerved cypselae and multiseriate pappus of persistent bristles suggested *Novenia*'s placement in subtribe Hinterhuberinae (Nesom 1994a).

DIOECY

Dioecy in Hinterhuberinae is present in the genus *Aztecaster* that includes two very similar, but widely allopatric species endemic to Mexico. *Aztecaster pyramidatus* Nesom was initially described as a species of *Bigelowia* DC. (Robinson and Greenman 1896) and also treated in *Chrysothamnus* Nutt. (Hall and Clements 1923) and *Haplopappus* Cass. (Blake 1926). Almost 100 years later Rzedowski (1972) discovered that this species was actually dioecious. Because dioecy in the tribe Astereae is present only in *Baccharis* L., *Archibaccharis* Heering and *Heterothalamus* Less. (subtribe Baccharidinae), he transferred *Bigelowia pyramidata* to *Baccharis*, and described a new species *Baccharis matudae* Rzed. In *Baccharis*, both species are anomalous in the morphology of flowers, phyllaries and cypselae, as summarized bellow:

Baccharis	*Aztecaster*
• phyllaries foliaceous, never completely indurate	• strongly indurate phyllaries
• zygomorphic pistillate corolla	• actinomorphic pistillate corolla
• staminate corolla lobes are cut to the base of the throat	• staminate corolla lobes cut about halfway to the base of the throat
• staminate style branches short with abruptly obtuse appendages	• staminate style branches long with tapering appendages
• cypselae 0-1.5 mm long, terete, or slightly compressed with 5-11 ribs or nerves, glabrous or moderately strigose,	• cypselae mostly 2.5-3 mm long, flat with two lateral nerves, moderately to densely strigose

Nesom (1993a) entertained the idea of close relationship of *Baccharis pyramidata* and *B. matudae* to *Ericameria* but their geographical isolation and the lack of dioecism within *Ericameria* did not favor such a connection (Nesom 1993a).

Nesom (1993a) hypothesized that dioecy in Baccharidinae and *Aztecaster* has developed along two different pathways. In Baccharidinae dioecious condition was preceded by a loss of fertility in the ovaries of the central flowers, followed by the loss of either pistillate or staminate flowers within a single head. The incomplete segregation of the sexes is found in the genus *Archibaccharis*. Pistillate heads in that genus almost always have few central staminate florets, while staminate heads rarely produce few peripheral pistillate florets, thus supporting the proposed evolutionary pathway of dioecy in Baccharidinae. In *Aztecaster* the capitula are either completely staminate or completely pistillate. Staminate flowers are morphologically and functionally similar to those of Baccharidinae. Pistillate flowers, however, may have derived from typically hermaphroditic ones by a reduction in size and the loss of stamens. Possibly *Aztecaster* was derived from an ancestor that produced discoid heads with only actinomorphic, hermaphroditic florets (Nesom 1993a). The tendency towards dioecy, in particular reduction in number and complete absence of central staminate flowers has been observed in *Diplostephium schultzii* Wedd. (Blake 1928, Cuatrecasas 1969) and in *Rochonia* (Humbert 1960).

BIOGEOGRAPHY

Reliable fossil data of Hinterhuberinae and in general, the entire family Asteraceae, are restricted to pollen. Because of the lack of any macrofossils, the place and the time of origin of Hinterhuberinae, and the entire family Asteraceae has been inferred from the present geographic distribution of the species and the geological history of the areas.

Estimates of the time of the origin of Asteraceae vary from Miocene to Cretaceous or earlier (Turner 1977). Recent estimates of divergence time based on rbcL and ndhF sequences suggested that Asteraceae originated in the mid Eocene (Kim et al. 2005). Pollen data indicate

that most of the tribes of the family were in existence by the end of Oligocene (22-25 my BP), a period that was followed by rapid evolution of the family and increase in species number probably in response to the worldwide aridization of the climate (Raven and Axelrod 1974). The place of origin for the family is hypothesized to be in the northern South America or northern Andes (Turner 1977, Raven and Axelrod 1974), in the Pacific area and South America, in particular Brazil, excluding the Amazon Basin (Bremer 1992), or in the southern South America (Stuessy et al. 1996).

Southern Hemisphere distribution of Hinterhuberinae (South America, southern Africa, Australasia and Madagascar) suggests that the ancestors of Hinterhuberinae were in existence at least by the early Tertiary, when the South Atlantic Ocean opened separating South America from Africa. Dispersal between the continents would have been possible via continental routes or across island bridges that existed between the continents until late Eocene - early Oligocene time (Tarling 1980). Direct migration of flora and fauna between South America and Australia through Antarctica was possible in Eocene and most likely continued through Oligocene, approximately some 35 my BP (Raven and Axelrod, 1972). Plant colonization of the puna and páramo zones of Andes was possible only after the final uplift of the mountains and cooling down of the climate, some 2-3 million years ago (Simpson 1975). Three processes have been suggested for the evolution of the diverse flora in the puna and páramo zone: 1) "vertical evolution", adaptation of lowland, warm-loving plants (neotropical elements) to high elevation environments, 2) long-distance dispersal of cool-adapted plants from north and south temperate regions, and 3) by speciation through isolation from autochthonous elements. Páramo and puna ecosystems in the form of "islands", are isolated from each other by low valleys which became more pronounced as the mountains

were progressively raised in the Tertiary, and have been, and still are, important barriers to dispersal of high altitude elements. The majority of colonization was hypothesized to have occurred during the glacial periods when plants were able to disperse more easily because of the increased size of the páramos and reduced distance between them (Simpson 1975). Geological and climatic history of the Andes provides strong support for a hypothesis of recent and rapid speciation and dispersal of the taxa in South American Hinterhuberinae. Because it is difficult to determine morphologically plesiomorphic character states within the subtribe Hinterhuberinae, the origin of Hinterhuberinae remains questionable. Biogeographical data suggest that the origin of Hinterhuberinae may have been in Madagascar, South America or Australasian regions (Nesom 1994a). Some apparently unspecialized genera do occur in these regions (Nesom 1994a).

RELATION WITH OTHER SUBTRIBES WITHIN TRIBE ASTEREAE

The relationship of Hinterhuberinae to the rest of the tribe is unclear. Presently, they have an unresolved position within the tribe Astereae, together with Baccharidinae, another primitively shrubby subtribe distributed primarily in the South America (Nesom 1994a). Several phylogenetic analyses imply Hinterhuberinae's polyphyletic nature (Noyes and Rieseberg 1999, Bayer and Cross 2002, Cross et al. 2002, Eastwood et al. 2002). In order to test hypotheses of the monophyly of Hinterhuberinae, the independent evolution of dioecy in Baccharidinae and Hinterhuberinae, and the South American origin of Hinterhuberinae and Astereae, the present molecular-based analyses of the nrDNA sequence data (external and internal transcribed spacers, ETS/ITS) will be used. In chapter 2 the relationship of *Hinterhubera* and related genera are investigated, as well as the evolution of dioecy in Hinterhuberinae and Baccharidinae. Chapter 3 discusses the relationship of *Chiliotrichum,*

Novenia, Celmisia and related genera, while chapter 4 investigates the monophyly of the subtribe Hinterhuberinae, its origin and the relationship of its members to other subtribes of Astereae. Finally, in chapter 5 preliminary phylogenetic analyses of matK sequence from cpDNA are presented.

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CHAPTER 2 PHYLOGENY OF *HINTERHUBERA* AND RELATED GENERA (HINTERHUBERINAE: ASTEREAE)

The informal *Hinterhubera* group within subtribe Hinterhuberinae was proposed by Nesom (1994a). Five of its seven constituent genera with approximately 22 species grow in the páramo and puna vegetation zones of the Andean Cordillera in South America, one genus of two species occurs in Mexico, and another with six species in Costa Rica and Panama (tab. 2.1). The group includes habitually diverse genera: *Flosmutisia* and *Floscaldasia* are herbaceous perennials, *Laestadia* includes small subshrubs and herbaceous perennials, *Westoniella* and *Hinterhubera* are mainly low shrubs with more or less densely tomentose, revolute, ericoid leaves, and *Parastrephia* and *Aztecaster* are tall shrubs with tomentose ericoid leaves. Additional defining features for the group include epaleate receptacle, disciform or discoid capitula, zygomorphic to actinomorphic tubular pistillate florets, with more or less reduced ligules (in *Floscaldasia* the ligules are well developed but the florets are minute and the capitula appear discoid), and functionally staminate, white, yellow, red or purple disk florets. *Aztecaster* is the only dioecious genus within Hinterhuberinae.

Hinterhubera, the type genus for the subtribe, has disciform capitula and deeply 4-5-lobed actinomorphic to zygomorphic peripheral female florets. Because of the disciform capitula it approaches the subtribe Conyzinae, but the tubular deeply lobed peripheral female florets differ from the filliform or shortly lobed peripheral florets in Conyzinae. Because of this distinctive floret features in combination with its low shrubby habit, Cuatrecasas (1969) recognized that *Hitnerhubera* could not be placed in any existing subtribe. Consequently, he established subtribe Hinterhuberinae (1969) and later revised it to include *Westoniella*, *Flosmutisia*, and *Blakiella* Cuatr. (Cuatrecasas 1986). Nesom (1994a) expanded the subtribe to

29 genera that he divided among seven informal groups encompassing about 460 species. Subsequently, the number of genera in Hinterhuberinae was increased to 33 with the reinstatement of *Blakiella*, *Paleaepappus* Cabrera, and *Aylacophora* Cabrera, addition of *Guynesomia* Bonif. & G. Sancho and *Printzia* Cass., and the exclusion of *Ericameria* Nutt. (Bonifancino and Sancho 2001, 2004, Urbatsch et al. 2003, Nesom and Robinson 2006).

Table 2.1. List of genera from the informal *Hinterhubera* group and their geographic distribution.

GENUS	# OF SPECIES	DISTRIBUTION
Hinterhubera Sch. Bip.	8	Venezuela, Colombia, N Chile
Parastrephia Nutt.	5	Peru, Chile, Argentina
Aztecaster Nesom	2	Mexico
Westoniella Cuatr.	6	Costa Rica, Panama
Laestadia Kunth	6	Venezuela, Colombia, Ecuador, Peru, Bolivia, Hispaniola, Costa Rica
Floscaldasia Cuatr.	2	Ecuador and Colombia
Flosmutisia Cuatr.	1	Colombia

Relationships among the genera under consideration have varied greatly (tab. 2.2). Long glandular hairs on corollas, disc florets with sterile ovaries and cypselae with glandular and twin-type trichomes, place *Flosmutisia* close to *Westoniella*, *Hinterhubera* and *Blakiella* (Cuatrecasas 1986). Highly modified peripheral corollas derived from central florets and flat cypselae ascertain close relationship of *Flosmutisia* and *Hinterhubera* (Nesom 1993b).

Westoniella is endemic to the páramos and subpáramos of Costa Rica and one species to the adjacent regions of Panama. Unlike tubular corollas in the subtribes Conyzinae, Baccharidinae and some Asterinae that were the result of extreme reduction of the ligulate corolla to a tube, zygomorphic tubular 5-toothed ray corollas of *Westoniella* were

hypothesized to be a primitive structure within the tribe Astereae itself. Hence, the genus was initially placed within the subtribe Asterinae (Cuatrecasas 1977), although similarity in habit and structure to other Andean genera (*Hinterhubera*, *Diplostephium* Kunth and *Blakiella*) was apparent. However, Cuatrecasas (1986) later transferred *Westoniella* to his redefined subtribe Hinterhuberinae.

Laestadia apparently is related to *Floscaldasia* and *Flosmutisia* that are also herbaceous Andean genera of low habit (Nesom 1994a). Historically, *Laestadia* was hypothesized to be closely related to *Lagenophora* Cass., *Solenogyne* Cass., *Myriactis* Less., and *Keysseria* Lauterbach (Cabrera 1966). Because of the similar structure of exothecial cells of the anthers, *Laestadia* was related to *Plagiocheilus* Arn. ex DC., *Lagenophora* and *Floscaldasia*, as part of the subtribe Grangeinae (Robinson and Bretel 1973). Later, *Laestadia* was excluded from Grangeinae (Fayed 1979) and tentatively placed within Bellidinae (Cuatrecasas 1986). Thick, entire, linear leaves and 6 to 10-nerved cypselae in *Laestadia*, suggest its relationship with highly specialized members of Hinterhuberinae, while the similarities with Grangeinae (epaleate receptacle, tubular pistillate ray florets, epappose cypselae) may have resulted from convergence (Nesom 1993b, 1994a).

Of taxonomic and historic note, there are two spelling variations of the name *Lagenophora*. Initially Cassini published the name with the spelling *Lagenifera* (Bull. Soc. Philom. Paris 1816), but later he considered the original spelling to be erroneous and corrected it to *Lagenophora* (Bull. Soc. Philom. Paris 1818). Since then both names have been adopted by various authors. The spelling herein follows the proposed conserved second and corrected spelling of the name (Hind and Jeffrey 1988, Nicolson 1996).

Table 2.2. Systematic position of genera from *Hinterhubera* group according to different authors.

GENUS	NESOM (1994A)	BREMER (1994)	CUATRECASAS (1969, 1986)	OTHERS
Hinterhubera	*Hinterhubera* group	*Hinterhubera* group (Asterinae)	Hinterhuberinae[a]	Baccharideae[1]
Parastrephia	*Hinterhubera* group	*Chiliotrichum* group (Asterinae)	-----	Baccharideae[1]
Aztecaster	*Hinterhubera* group	*Erigeron-Conyza* group (Asterinae)	-----	---------
Westoniella	*Hinterhubera* group	*Hinterhubera* group (Asterinae)	Hinterhuberinae[b]	Asterinae[3]
Laestadia	*Hinterhubera* group	*Lagenophora* group (Asterinae)	Grangeinae[a], Bellidinae[b]	Baccharideae[1], Grangeinae[2]
Floscaldasia	*Hinterhubera* group	*Lagenophora* group (Asterinae)	Asterinae[a]	Grangeinae[2]
Flosmutisia	*Hinterhubera* group	*Hinterhubera* group (Asterinae)	Hinterhuberinae[b]	-----

[1]=Weddell (1855); [2]=Robinson and Brettell (1973); [3]=Cuatrecasas (1977).
[a]= Cuatrecasas (1969); [b]=Cuatrecasas (1986)

Floscaldasia is remarkably similar in its creeping habit and reduced leaves to *Laestadia*, and it was often misidentified as such (Nesom 1993b; Sklenář and Robinson 2000). Its pappus bristles and lack of cypselae beak distinguish it from *Laestadia*. *Floscaldasia* had been considered a member of three different subtribes: Asterinae (Cuatrecasas 1969), Grangeinae (Robinson and Brettell 1973) or Bellidinae (Cuatrecasas 1986). Bremer (1994) accepted Robinson and Brettell's hypothesis of close relationship of *Laestadia* and *Floscaldasia*, but kept these two genera in his *Lagenophora* group, within subtribe Asterinae. Currently, the genus resides in Hinterhuberinae, among the most

specialized elements of the subtribe together with *Laestadia*, *Westoniella* and *Flosmutisia* (Nesom 1994a, Nesom 2000).

One of the genera with controversial position within the subtribe is *Parastrephia.* Initially discoid *Parastrephia* was considered closely related to *Lepidophyllum* Cass., a genus with radiate capitula from the informal *Chiliotrichum* group (Nesom 1993c, Cabrera 1945) or treated as synonym of that genus (Bentham and Hooker 1873, Hoffman 1890). However, the species of *Parastrephia* distributed in the puna region of Bolivia and surrounding areas in Peru, Chile and Argentina are markedly different from *L. cupressiforme* Cass., a species restricted to Patagonia, in having alternate leaves (versus opposite leaves in the latter), tubular or shortly ligulate marginal florets (versus well developed ligules), 2-4 nerved cypselae (versus multinerved cypselae), and pappus of capillary bristles (versus flattened, subpaleate bristles). Because of these morphological differences, Cabrera (1954) reinstated *Parastrephia* recognizing five species within it. Two of those species, *Parastrephia quadrangularis* and *P. lepidophylla* differ only in size, stem thickness and phenology. Plants with transitional characters between the two species are common (Nesom 1993c). Similarly, *P. phylicaeformis* differs from *P. lucida* in having more tomentose stem, and plants with intermediate features between the two can be found. Although Cabrera (1978) noted the weakness of these characters, he decided to keep the species as separate entities. However, taking into account the presence of intermediate forms between the species, Nesom (1993c) proposed that *P. phylicaeformis* and *P. lepidophylla* be reduced to the synonyms of *P. lucida* and *P. quadrangularis* respectively.

Dioecy in Hinterhuberinae is present in *Aztecaster* which includes two very similar, but widely allopatric species endemic to Mexico. *Aztecaster pyramidatus* was first described

as a species of *Bigelowia* DC. (Robinson and Greenman 1896), following Gray's (1884) broad concept of that genus. In their treatment of *Chrysothamnus* Nutt., Hall and Clements (1923) removed several species from *Bigelowia*, including *B. pyramidata* Robinson & Greenm., and placed it within *Chrysothamnus*, in their section *Nauseosi*, because of its pannose tomentum on the twigs. The relationship with other species of the section was not clear, but they hypothesized that it was probably basally divergent in that lineage. Blake (1926) subsequently placed the species in the large genus *Haplopappus* Cass. close to *Ericameria*. At that time and until 1972 when Rzedowski discovered that "*Bigelowia pyramidata*" or what is now *Aztecaster pyramidatus* was actually dioecious, the species characterizations had been based only on staminate plants. Because dioecy in the tribe Astereae is present only in *Baccharis* L., *Archibaccharis* Heering and *Heterothalamus* Less. (subtribe Baccharidinae) he transferred "*Bigelowia pyramidata*" to *Baccharis*, and at the same time described a new species, *Baccharis matudae* Rzed. However, within *Baccharis* both species were anomalous in their floral, phyllary and cypselae morphology (see chapter 1). Alternatively, *Aztecaster pyramidatus* and *A. matudae* were considered similar to *Ericameria* in their shrubby habit, production of resins, presence of pannose tomentum, narrow ericoid leaves, discoid heads, morphology of style branches in staminate florets and cypselae structure. Their placement in *Ericameria* too was tenuous because the latter radiated in northwestern Mexico and the western United States, and lacked any tendency towards dioecy (Nesom 1993a).

Dioecy in Baccharidinae and *Aztecaster* probably developed along two different pathways (Nesom 1993a). In Baccharidinae dioecious condition has been preceded by loss of fertility in the ovaries of the central florets, followed by the loss of either pistillate or staminate florets within a single head. The incomplete segregation of the sexes is found in the

genus *Archibaccharis*. Pistillate heads in that genus almost always have few central staminate florets, while staminate heads rarely produce few peripheral pistillate florets, thus supporting the proposed evolutionary pathway of dioecy in Baccharidinae. In *Aztecaster* heads are either completely staminate or completely pistillate. Staminate florets are morphologically and functionally similar to those of Baccharidinae. Pistillate florets, however, may have been derived from typically hermaphroditic ones by a reduction in size and loss of the stamens. There is a tendency for the reduction of ligulate florets in Hinterhuberinae (*Parastrephia*, *Hinterhubera*, *Westoniella*). Tendency towards dioecy, particularly reduction in number and complete absence of central staminate florets, has been observed in *Diplostephium schultzii* Wedd. (Blake 1928, Cuatrecasas 1969) and in *Rochonia* DC. (Humbert 1960). Hence, ovarian sterility in *Aztecaster* probably has been inherited from the ancestor that probably produced discoid heads with only actinomorphic, hermaphroditic florets (with fertile ovaries), and was missing peripheral, ligulate, pistillate florets (Nesom, 1993a).

Because of widespread morphological similarities in the tribe Astereae, postulated hypotheses of the relationship of genera within the informal *Hinterhubera* group may be the result of convergent evolution. Molecular analyses of the ITS sequence data (Noyes and Rieseberg 1999, Cross et al. 2002, Eastwood et al. 2004) did not include any representative from the informal *Hinterhubera* group. Thus the postulated hypotheses of their relationships within and among other groups have remained untested. Present study of the ITS and ETS sequence data (see chapter 4) postulate derived position of the informal *Hinterhubera* group within Hinterhuberinae and its closer relation to South American taxa from other subtribes with primary Southern Hemisphere distribution.

The objectives of the present study were to: 1) test the monophyly of the *Hinterhubera* group; 2) test the monophyly of the genera within the group, 3) use molecular estimate of phylogeny to evaluate the hypothesis of the evolution of dioecy in Hinterhuberinae.

MATERIALS AND METHODS

Taxon Sampling. Sampling was designed to include as many taxa from Nesom's (1994a) informal *Hinterhubera* group as possible. From a total of 7 genera and 30 species that he recognized, 6 genera and 20 species were sampled. The monotypic genus *Flosmutisia* was not included because plant material was unavailable. In order to test taxonomic changes within *Parastrephia* postulated by Nesom (1993c), all five Cabrera's (1954) species were sampled. Extractions were done from leaf material removed from herbarium specimens, or plant material dried in silica gel crystals. Herbarium material was obtained from the following herbaria: F, LSU, MERC, MO, NO, NY, QCA, QCNE, SGO, TEX/LL, US, and USM, (acronyms follow the Index Herbariorum, New York Botanical Garden. http://sciweb.nybg.org/science2/IndexHerbariorum.asp). Silica dried material was kindly provided by M. Bonifancino (Facultad de Agronomia, Laboratorio de Botanica, Montevideo, Uruguay). Identification of the species was confirmed using keys published in regional floras (Black 1957, Allan 1961, Aristeguieta 1964, Koster 1966, Cuatrecasas 1969, Cabrera 1971, Stanley and Ross 1983, Wagner et al. 1990, Herman et al. 2000) and other taxonomic works (Cabrera 1945, 1954, 1966, Cuatrecasas and Aristeguieta 1956, Humbert 1960, Wild 1969, Jackson 1975, Cuatrecasas 1977, 1986, Dillon 1981, Nesom 1993a, 1993c, 1994b, Bonifancino and Sancho 2001, 2004).

The choice of the genera included in the data set was based on the previous morphological (Cuatrecasas 1986, Nesom 1994a, 2000, Nesom and Robinson 2006) and

molecular analyses (Noyes and Rieseberg 1999, Lowrey et al. 2001, Cross et al. 2002, Fiz et al. 2002, Eastwood et al. 2004). To investigate the relationship of the group within the tribe Astereae, one to eight genera from subtribes primarily distributed in the Southern Hemisphere were included in the dataset: Felicinae, Grangeinae, Podocominae, Brachycominae, Lagenophorinae, and Baccharidinae. In addition representatives from Asterinae and Bellidinae, the genera with primarily Old World distribution, were sampled. *Doellingeria* Nees and *Eucephalus* Nutt., were chosen as representatives of the North American clade *sensu* Noyes and Rieseberg (1999) because of their basal position in this lineage. Selection of genera from the rest of the subtribe Hinterhuberinae was based on the molecular analyses presented in chapter 4. Hence, *Diplostephium* (*Chiliotrichum* group), *Madagaster* Nesom, *Olearia* Moench and two currently unplaced genera (*Blakiella* and *Guynesomia*), were also included. *Olearia*, a large polyphyletic genus, was presented with one species, the choice of which was based on the results obtained by Cross et al. (2002).

Amellus L., subtribe Felicinae, was selected as the outgroup based on the molecular analyses of Noyes and Rieseberg (1999) and Cross et al. (2002). In both investigations Felicinae, represented by *Felicia* Cass. and *Amellus*, were resolved as an early diverging lineage of Astereae basal to the *Hinterhubera* group and other genera of interest.

Character Sampling. This research employed the analysis of the following data sets: internal transcribed spacer (ITS) and external transcribed spacer (ETS) of nuclear ribosomal DNA and the combined ITS/ETS data set. The ITS region has been successfully used for phylogenetic analysis of Asteraceae on generic and species level (Baldwin et al. 1995, Morgan 1997, Noyes 2000, Urbatsch et al. 2000, 2003, Lowrey et al. 2001, Cross et al. 2002, Plovanich and Panero 2004, Martins and Hellwig 2005). The ETS has also been proven to be

well suited for resolving phylogenies at generic and species level (Baldwin and Markos 1998, Linder et al. 2000, Markos and Baldwin 2001). In Asteraceae, this region is considerably longer than ITS-1 and ITS-2, and has greater sequence variation; it is 1.5 times more informative than ITS region (Bena et al. 1998, Baldwin and Markos 1998, Linder et al. 2000, Markos and Baldwin 2001). However, because ETS and ITS are part of the same transcription unit, the ETS region cannot be considered completely independent from the ITS region, but it can serve to augment the ITS data (Baldwin and Markos 1998, Markos and Baldwin 2001). Consequently, sequence data for ITS and ETS regions were used independently and in combination to successfully investigate relationship within and among the species of *Calycadenia* DC. (Baldwin and Markos 1998), *Silphium* L. (Clevinger and Panero 2000), *Xylothamia* Nesom, Suh, D. Morgan & Simpson and *Gundlachia* Gray (Urbatsch et al. 2003), *Tragopogon* L. (Mavrodiev et al. 2005), *Machaeranthera* Nees (Morgan 2003), to mention few. The analyses of combined datasets provided better resolution of trees and higher bootstrap support for monophyletic groups than analyses of ITS or ETS sequence data alone (Baldwin and Markos 1998, Linder et al. 2000, Markos and Baldwin 2001). Accordingly, the ITS and ETS sequence data in this study were combined.

The ITS data matrix included 87 accessions representing 43 genera and 82 species; 54 sequences were presented for the first time, and the remaining 33 were obtained from the GenBank. The ETS data matrix included 60 accessions representing 31 genera and 56 species; 58 accessions were presented for the first time and two were acquired from the GenBank. The taxon sampling between ITS and ETS data sets could not be matched exactly because of difficulties in amplifying either the ETS or the ITS region in number of taxa. Therefore the

combined data set included a total of 55 accessions from 31 genera and 51 species. Species, vouchers and accession numbers are given in table 2.3.

DNA Extraction, Amplification (PCR) and Sequencing. Each species was sampled from two different populations, when possible. Approximately 20-50 mg of dry leaf material was ground using the Mini Beadbeatter 8 (BioSpec Products, Bartlesville, OK). Total genomic DNA was extracted and purified using DNeasy Plant Mini kit (Qiagen, Valencia, CA) or MasterPure™ Plant Leaf DNA Purification kit (Epicentre Biotechnologies, Madison, WIS) following manufacturers' protocols with some modifications. In both cases, ground tissue was soaked in extraction buffer for 1 h at 65°C or 70°C, instead of the recommended 10 to 30 min. When Epicentre's kit was employed, grinding was preceded by soaking leaf material in 10 μM EDTA buffer for approximately 1 hour to facilitate extraction of the DNA from the cells. Excess of buffer was removed with Kim-Wipes and grinding of leaves was carried out by alternating the cycles of freezing the tissue by placing the tubes in liquid nitrogen, and grinding for 15-20 s. In addition, extracted DNA was left to precipitate overnight in isopropanol at -20°C. The remaining steps followed the manufacturer's protocol. Total genomic DNA was visually quantified by electrophoresing the ethidium-bromide stained samples on 2% agarose gels.

Amplifications were performed in 25 μl volumes containing 2 μl of DNA template, 12.5 μl of MasterAMP™ 2x PCR Premix GN (Epicentre Biotechnologies, Madison, WIS), 8 μl of distilled water, 0.5 μl MasterAMP™ Tfl DNA Polymerase (Epicentre Biotechnologies) and 1 μl of each 10 nM forward and reverse primer. Initial preheating of the reaction mixture

Table 2.3. List of taxa, collection information and GenBank numbers. References for sequences obtained from other sources are given.

SPECIES LIST	LOCALITY	COLLECTOR (HERBARIUM)	GENBANK # ITS	GENBANK # ETS	REFERENCE
Subtribe Asterinae					
Aster amellus L.	Austria: Wien	A. Heimerl (LSU)		DQ479042	
Aster amellus L.	Russia: N. Caucasus	A. K. Skvortsov s.n.	AF046961		Noyes & Rieseberg 1999
Bellidiastrum michelii Cass.	France: Valais: Bourg. St. - Pierre	S. Castroviejo 11118 (MA)	AF494011		Fiz et al. 2002
Galatella lynosiris Rchb. f. [1]	Russia: Saratov.	A.K. Skvortsov s.n. (MO)	AF046949		Noyes & Rieseberg 1999
Galatella linosyris Rchb. f. [2]	Austria	Seiller s.n (F)	DQ478987	DQ479043	
Galatella coriacea Novopokr.	Uzbekistan: Tashkent, Bostandyskskiy	Korovina (MA240960)	AF494003		Fiz et al. 2002
Kalimeris pinnatifida (Maxim.) Kitam.	cult. USA: S. Carolina: Columbia	Porter s.n. (USCH)	DQ478988	DQ479044	
Subtribe Baccharidinae					
Archibaccharis androgyna Blake	Mexico: Chiapas: Cerro Mozatal	D. E. Breedlore 3116 (F)	DQ478989	DQ479045	
Archibaccharis asperifolia (Benth.) Blake [1]	Guatemala: Quiche: Sierra Madre. 1800-2000 m alt.	L. O. Williams 41606 (F)		DQ479046	
Archibaccharis asperifolia (Benth.) Blake [2]	Mexico: Hidalgo: Apulco-Agua Blanca, 2100 m alt.	R. Hernandez M. 4084 (MO)	DQ478990	DQ479047	
Archibaccharis schiedeana (Benth) J. D. Jackson	Mexico: Comala: Volcan de Colima; 1370 m alt.	E. J. Lott 3035 (MO)	DQ478991		
Baccharis boliviensis (Wedd) Cabrera	Argentina: San Louis: Coronel Pringles; 1532 m alt.	R. H. Fortunato 5518 (USA)	DQ478992	DQ479048	
Baccharis dracunculifolia DC.	Bolivia: La Paz	M. Lewis 35355 (MO)	AF046958		Noyes & Rieseberg 1999
Baccharis neglecta Britton.	USA: Texas: Travis Co.	Morgan 2055 (TEX)	U97604		Morgan 1997
Heterothalamus spartioides Hook. & Arn.	Argentina: Rio Negro: Adolfo Alsina.	G. Seijo 1454 (NY)	DQ478993	DQ479049	

table 2.3 continued

Subtribe Bellidinae					
Bellis perennis L.	Bolivia: La Paz	J.C. Solomon 8238 (MO)	AF046950		Noyes & Rieseberg 1999
Subtribe Brachycominae					
Brachycome basaltica F. Muell. var. *gracilis* Benth.	Australia: Oxley	P. G. Kodela 453 (MO)		DQ479050	
Brachycome ciliaris Less.	W. Australia	T. Lowrey 1712 (UNM)			Lowrey et al 2001
ITS1 ITS2	"	"	AF247067 AF247068		"
Brachycome rigidula (D.C.) G. L. Davis	Australia: Victoria: Mt. Hotham; 1700-1800 m alt.	A. Strid 22025 (MO)	DQ478994	DQ479051	
Subtribe Felicinae					
Amellus microglossus DC.	South Africa: Oranjemund: N. W. Cape; 700 m alt.	M. F. Thompson 159 (MO)	DQ478995	DQ479052	
Amellus strigosus (Thunb.) Less.	South Africa: E Cape: Port Elizabeth; 20 m alt.	K. A. Dahlstrand 3153 (MO)	DQ478996	DQ479053	
Felicia aethiopica (Burm. f.) Bol. & W. Dod	South Africa: Cape Province	E. Esterhysen 36321(MO)	DQ478997	DQ479054	
Felicia echinata Nees	South Africa: Eastern Cape	(E)	AY193797		Eastwood et al. 2004
Felicia fruticosa Nichols. ssp. b*revipedunculata* (Hutchinson) Grau	South Africa: Cape Province: Victoria West District; 1300 m alt.	I. H. Hartley 873	AY193795		Eastwood et al. 2004
Zyrphelis decumbes (Schltr.) Nesom	South Africa: Worcester Div.: Old Toll Gate	F. M. Leighton 1978 (NY)	DQ478998	DQ479055	
Subtribe Grangeinae					
Grangea maderaspatana (L.) Poir	Sri Lanka: Northwestern Province Kurunegala District; 300 m alt.	D. D. Socjarto 4840 (F)		DQ479056	
Grangea maderaspatana (L.) Poir.	Thailand: Chiang Mai	J.F. Maxwell 90-218 (MO)	AF046951		Noyes & Rieseberg 1999
Nidorella polycephala DC.	South Africa: Town Hill: Natal	J. L. Sidey 3023 (F)	DQ478999	DQ479057	

table 2.3 continued

Nidorella resedifolia DC.	Transvaal: Zoutpansberg; 1800 m alt.	M. J. Schlieben 7318 (F)	DQ479000	DQ479058	
Plagiocheilus bogotensis (H.B.K.) Weddell	Ecuador: Pichincha; 3180 m alt.	V. Zak 86 (MO)	DQ479001	DQ479059	
Plagiocheilus solivaeformis DC.	Peru: Cajamarca: Cajamarca; 3550 m alt.	M. Dillon et al. 2937 (F)	DQ479002	DQ479060	
Subtribe Hinterhuberinae					
***Chiliotrichum* group**					
Diplostephium rupestre (H.B.K.) Wedd.	Ecuador: Napo	L. Holm-Nielsen 28233 (MO)	AF046962		Noyes & Rieseberg 1999
Diplostephium ericoides (Lam.) Cabrera [1]	Ecuador; Cotopaxi-Napo border; 3800-3850 m alt.	J. L. Luteyn 13438 (LSU)	DQ479003	DQ479061	
Diplostephium ericoides (Lam.) Cabrera [2]	Ecuador: Carchi	R. M. King et all. 10115 (F)	DQ479004	DQ479062	
***Hinterhubera* group**					
Aztecaster matudae (Rzed.) Nesom [1]	Mexico: Coahuila, Sierra Zapaliname; 2480 m alt.	G. B. Hinton et al. 20225 (TEX)	DQ479005		
Aztecaster matudae (Rzed.) Nesom [2]	Mexico: Neuvo Leon; 2140 m alt.	Vankat 98 (TEX)	DQ479006	DQ479063	
Aztecaster pyramidatus (B.L.Rob. & Greenm.) Nesom [1]	Mexico: Oaxaca; 1600 m alt.	L. Woodruff 227 (TEX)	DQ479007		
Aztecaster pyramidatus (B.L.Rob. & Greenm.) Nesom [2]	Mexico: Oaxaca: Monte Alban; 1850 m.	J. Rzedowski 28828 (TEX)	DQ479008	DQ479064	
Floscaldasia hypsophila Cuatr.	Ecuador: Azuay: Cajas National Park; 4400 m alt.	P. Sklenar 2418 (USA)	DQ479009	DQ479065	
Hinterhubera adenopetala Cuatr. & Aristeg.	Venezuela: Trujillo: Parque Nacional Dinira; 3300 m alt.	R. Riina et al. 749 (USA)	DQ479010	DQ479066	
Hinterhubera columbica Sch. Bip. ex Wedd.	Venezuela: Merida: Sierra Nevada de Santo Domingo; 3370-3380 m alt.	J. Cuatrecasas 28087 (F)	DQ479011	DQ479067	
Hinterhubera ericoides Weddell [1]	Venezuela: Merida: Serra Nevada; 3900-4050	Bernardi 16996 (F)	DQ479012	DQ479068	

table 2.3 continued

Hinterhubera ericoides Weddell [2]	Venezuela: Merida: Sierra Nevada: Paramo Media Luna; 4190 m. alt.	A. Berg 189 (MERC)	x	x
Hinterhubera imbricata Cuatr. & Aristeg.	Venezuela: Merida: Parque Nac. Sierra Nevada; 4170 m alt.	A. Berg 171A (MERC)	DQ479013	DQ479069
Hinterhubera lanuginosa Cuatr. & Aristeg.	Venezuela: Merida: Distrito Rangel: Paramo de Mucuchie	S. E. Carpenter 893 (NY)	DQ479014	DQ479070
Hinterhubera laseguei Wedd.	Venezuela: Merida: Paramo Las Cruces; 4180 m alt.	P. E. Berry 3953 (USA)	DQ479015	DQ479071
Laestadia costaricensis Blake	Costa Rica: Cartago: Cordillera de Talamanca; 9100 ft. alt.	R. L. Wilbur 9971 (F)	DQ479016	DQ479072
Laestadia muscicola (Sch. Bip.) Wedd. [1]	Colombia: Antiquia: Paramo de Frontino; 3320-3450 m alt.	J. M. MacDougal 4460 (USA)		DQ479073
Laestadia muscicola (Sch. Bip.) Wedd. [2]	Venezuela: Barinas: Paramo El Toro; 2480 m alt.	Briceño, B. 2238 (MERC)	DQ479017	DQ479074
Laestadia pinifolia Kunth	Colombia: Boyaca: Paramo de Chita; 3140 m alt.	A. M. Cleef 4754	DQ479018	DQ479075
Parastrephia lepidophylla (Wedd.) Cabr. [1]	Peru: Arequipa: Cailloma; 3542 m alt.	J. Treacy 822 (F)	DQ479019	DQ479076
Parastrephia lepidophylla (Wedd.) Cabr. [2]	Peru: Moquegua: Mariscal Nieto; 3680 m alt.	M.O. Dillon 4810 (TEX)	DQ479020	
Parastrephia lucida (Meyen) Cabrera [1]	Bolivia: Potosi: Sud Lipez; 4670 m alt.	E. Garcia 1102 (USA)	DQ479021	DQ479077
Parastrephia lucida (Meyen) Cabrera [2]	Bolivia: Oruro: Carangas ; 3920 m alt.	B. Vuilleumier 310 (TEX)	DQ479022	DQ479078
Parastrephia phylicaeformis (Meyen) Cabrera	Argentina: Jujuy: Mina La Esperanza; 4600 m alt.	V. A. Funk 11158 (USA)	DQ479023	DQ479079
Parastrephia quadrangularis (Meyen) Cabrera [1]	Peru: Moquegua: Mariscal Nieto; 3600 m alt.	M. O. Dillon 343 (TEX)	DQ479024	DQ479080
Parastrephia quadrangularis (Meyen) Cabrera [2]	Bolivia: La Paz: Pacajes: Caquiaviri; 4200 m alt.	T. Johns 82-30 (F)	DQ479025	DQ479081
Parastrephia teretiuscula (Kuntze) Cabrera	Bolivia: Potosi: Antonio Quijarro; 3700 m alt.	G. Torrico 507 (USA)	DQ479026	DQ479082

table 2.3 continued

Westoniella chirripoensis Cuatr.	Costa Rica: Cartago: Cerro de la Muerte.	V. Karaman-Castro 237 (LSU)	DQ479027	DQ479083	
Westoniella eriocephala (Klatt) Cuatr.	Costa Rica: San José: Cerro Chirripó; 3500 m alt.	G. Davidse 1545 (USA)	DQ479028	DQ479084	
Westoniella kohkemperi Cuatr.	Costa Rica: Cerro Truncado, 3700 m alt.	A. S. Weston 10093A (USA)	DQ479029	DQ479085	
Westoniella triunguifolia Cuatr.	Costa Rica: San Jose: Cerro de la Muerte.	V. Karaman-Castro 239 (LSU)	DQ479030	DQ479086	
***Madagaster* group**					
Madagaster madagascariensis (Humbert) Nesom	Madagascar: Central Madagascar: Mt. Ibity; 1700 m alt.	B. DuPuy MB302 (MO)	DQ479031	DQ479087	
Madagaster mandrarensis (Humbert) Nesom	Madagascar: Antsiranana Reserve Naturelle Marsojejy; 1100-1300 m alt.	J. S. Miller 3525 (MO)	DQ479032	DQ479088	
Mairia lasiocarpa DC.	South Africa: Swellendam - Distrikt; 900 m alt.	R. Schlechter 5582 (MO)		x	
***Olearia* group**					
Olearia arguta Benth.	Queensland	Cumming 13918 (BRI)	AF247066		Lowrey et al. 2001
Olearia ramulosa (Labill.) Benth	Australia: Victoria: Grampians National Park Area; 370 m alt	R. M. King 9722 (F)	DQ479033	DQ479089	
***Remya* group**					
Remya kauaiensis Hillebrand	USA: Hawaii: Kauai	1996249 (BISH)	AF497684		Cross et al. 2002
Remya mauiensis Hillebrand	USA: Hawaii: Mauai	K. R. Wood 7317 (BISH)	AF497685		Cross et al. 2002
unplaced					
Blakiella bartsiaefolia (Blake) Cuatr.	Venezuela: Merida: Rangel; 3000 m alt.	H. van der Werff 7707 (USA)	DQ479034	DQ479090	
Guynesomia scoparia (Phil.) Bonif. & G.Sancho	Chile: Elqui: Llanos de Huanta.	M. Bonifancino 820 (LP)	DQ479035	DQ479091	

table 2.3 continued

Subtribe Lagenophorinae					
Keysseria maviensis (H. Mann) Cabrera [1]	USA: Hawai: Maui: Puu Kukui trail. 5000 ft. alt.	S. Perlman 6875 (USA)	DQ479036	DQ479092	
Keysseria maviensis (H. Mann) Cabrera [2]	USA: Hawai: Mau: Kahoolewa Ridge; 5280 ft. alt	S. Perlman 10579 (F)		DQ479093	
Lagenophora pumila Cheeseman	New Zealand: South island: Boulder Lake Trail.	S. A. Norton 598 (NO)	DQ479037	DQ479094	
Myriactis humilis Merr.	Taiwan: Pingtum Hsien	T. Chiang 141 (MO)	AF046959		Noyes & Rieseberg 1999
Myriactis panamensis S.F. Blake	Panama: Chiriqui	R. J. Schmalzel 1713 (MO)	AF046965		Noyes & Rieseberg 1999
Subtribe Podocominae					
Kippistia suaedifolia F. Muell.	New South Wales	Pickard 3657 (NSW)	AF497660		Lowrey et al. 2001
Laennecia schiedeana (Less.) G.L.Nesom	Mexico: Oaxaca: Miahuatlan; 2260 m alt.	Hinton s.n. (LSU)	DQ479038	DQ479095	
Laennacia sophiifolia (Kunth) Nesom	Mexico: Oaxaca.	L. L. Lopez 346 (MO)	AF046964		Noyes & Rieseberg 1999
Minuria integerrima (DC.) Benth.	Queensland	T. Lowrey 1754 (UNSW)	AF247074		Lowrey et al. 2001
Minuria macrorhiza (DC.) Lander	Northern Territory	Adam 23307 (UNSW)	AF247076		Lowrey et al. 2001
Peripleura bicololor (Burbidge) Nesom	Queensland	T. Lowrey 1765 (UNSW)	AF247078		Lowrey et al. 2001
Peripleura diffusa (Burbidge) Nesom	Queensland	T. Lowrey 1760 (NSW)	AF247079		Lowrey et al 2001
Podocoma notobellidiastrum (Griseb.) Nesom	Paraguay: Caazapa	E. Zardini 3009 (MO)	AF046963		Noyes & Rieseberg 1999
Sommerfeltia spinulosa Less.	Uruguay: Salto: Cerros del Boquerón de Ararunguá.	T. M. Pedersen 16281 (NY)	DQ479039	DQ479096	
Tetramolopium humile (A. Gray) Hillebr. ssp. *humile* var. *humile*	USA: Hawaii: Hamakua: Mauna Kea; 9400 ft alt.	T. K. Lowrey 436 (MO)	DQ479040	DQ479097	

table 2.3 continued

Tetramolopium pumilum Mattf.	New Guinea	T. Lowrey 1546 (UNM)	AF247092		Lowrey et al. 2001
Tetramolopium remyi (A. Gray) Hillebr.	USA: Hawaii	T. Lowrey 449, 450 (UC)	AF247093		Lowrey et al. 2001
Tetramolopium vagans Pedley	Queensland	T. Lowrey 1755 (UNSW)	AF247096		Lowrey et al. 2001
Vittadinia sulcata Burbidge	W. Australia	T. Lowrey 1727 (UNSW)	AF247112		Lowrey et al. 2001
Unknown Subtribe					
Commidendrum rotundifolium (Roxb.) DC.	St. Helena: Pounceys.	(E)	AY193793		Eastwood et al. 2004
Commidendrum spurium (G. Forst.) DC.	St. Helena: Mount Vesey; 700 m alt.	(E)	AY193790		Eastwood et al. 2004
Doellingeria umbellata (Mill.) Nees	USA: Michigan: Chippewa Co.	Schmidt & Merello 1060 (TEX)	AF477625	AF477754	Urbatsch et al. 2003
Eucephalus glabratus (Greene) Greene	USA: California: Siskiyon Co.: Scott Mountains.	V. Karaman 122 (LSU)	DQ479041	DQ479098	
Melanodendron integrifolium (Roxb.) DC.	St. Helena: High Peak; 720 m alt.	V. Thomas 1 (E)	AY193794		Eastwood et al. 2004

at 95°C for 1 min was followed by 31 cycles of amplification as follows: (i) 10 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min with an additional 4 s at each cycle; and (ii) 21 cycles of denaturation at 95°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 1 min 40 s, extended for 4 s each cycle. A final extension period of 7 min at 72°C finished the amplification. All amplifications were performed in PTC-100™ Thermal Cycler (MJ Research, Inc., Watertown, MAS). This protocol was used for amplification of both, ETS and ITS regions.

Amplification of the ITS region (including the ITS-1 and ITS-2 spacer regions and the 5.8S ribosomal subunit) was routinely done in two successive PCR reactions. The first reaction used a set of external primers, ITS-18 and ITS-350 (Urbatsch et al. 2000) or ITS-18 and ITS-4 (White et al. 1994). The amplicons from the first reactions were directly used as templates in the second amplification with nested primers ITS-I (Urbatsch et al. 2000) and ITS-4. In some cases a modification of ITS-I, the ITS-I.1 (Roberts 2002) was used. If these sets of primers did not work, a combination of ITS-20, ITS-262 (Urbatsch et al. 2000), and above mentioned primers was implemented.

The ETS region was amplified using primers Ast-8 (Markos and Baldwin 2001) and 18S-ETS (Baldwin and Markos 1998). If this initial amplification yielded no results or the concentration of amplicons was insufficient for sequencing, the amplicons were used as templates in the second PCR reaction done with nested primer Ast-1 (Markos and Baldwin 2001) and 18S-ETS. In most of the cases, the second PCR reaction was preceded by purification of the template using QIAquick PCR Purification columns (Qiagen Inc.).

Excess primer and unincorporated nucleotides were removed from the PCR products by spin column centrifugation (QIAquick PCR Purification kit, Qiagen Inc.) following the

manufacturer's protocol. Prior to sequencing, quantification of the PCR products was performed visually on 2% agarose gels using Low Mass DNA Ladder (Life Technologies, Rockville, MA). Sequencing was performed directly using BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) in 10 µl reactions following manufacturer's protocol. The ITS region, including ITS-1, 5.8S subunit and ITS-2, was sequenced using ITS-I or ITS-I.1 and ITS-4 primers, and the ETS region using 18S-ETS and either Ast-8 or Ast-1 primers. The cycle sequencing protocol consisted of 26 cycles of denaturation at 96°C for 10 s, annealing at 50°C for 5 s and extension at 60°C for 4 min. Cycle sequencing reactions were initially purified by ethanol precipitation and ran on an ABI 377 automated sequencer (Applied Biosystems, Foster City, CA). Later analyzed samples were purified using Sephadex columns and run on an ABI PRISM 3100 (Applied Biosystems, Foster City, CA). Automated sequencing was done at the Museum of Natural Sciences molecular laboratory, Louisiana State University, Baton Rouge, LA.

Sequence Alignment and Analysis. Sequences were edited in Sequencher™ 4.1 (Gene Codes Corporation, Ann Arbor, MI). Initial sequences were aligned using Clustal X ver. 1.81 (Thompson et al. 1997) and manually adjusted in MacClade 4.08 (Maddison and Maddison 2000) as needed. New sequences were added to the existing matrices and aligned visually. The final aligned data matrices were subjected to simple neighbor-joining analyses. Obtained trees were then used to order the taxa according to their phylogenetic similarity, and then realigned manually, thus minimizing the homoplasy. The boundaries of the ITS and ETS regions were determined using the published sequences available in the GenBank (Noyes and Rieseberg 1999, Markos and Baldwin 2001).

Phylogenetic Reconstruction. Maximum parsimony and Bayesian analyses were performed for ITS and ETS data sets, independently, and for the reduced combined data set. The two data sets were tested for their combinability using the Partition homogeneity test (the incongruence length difference test of Farris et al. 1994) incorporated in Paup* 4.0b10 (Swoford 2000). The partition homogeneity test was implemented using TBR branch-swapping algorithm simple addition of sequences and saving 200 trees at each replicate.

Maximum parsimony (MP) was performed using Paup* 4.0b10 (Swoford 2000). Heuristic searches used random stepwise taxon addition to obtain starting trees and the tree bisection-reconnection (TBR) branch swapping option, while keeping the steepest descent option off. Nucleotide characters were unordered and equally weighted, and gaps were treated as missing data. Searches were replicated 10,000 times. Heuristic search of the datasets was conducted in two steps due to the memory limitations. The initial searches were performed saving 100 (for ITS and combined data sets) or 50 (for ETS data set) trees per replicate in order to explore different tree islands. These heuristic searches resulted in a pool of trees that were then used as starting trees in a second heuristic search where the trees were swapped to completion (Maddison 1991). Relative support for the clades was calculated using bootstrap analyses with TBR branch swapping, 1000 bootstrap replicates each with 10 random addition replicates, and saving 100 trees per replicate (Mort et al. 2000, Salamin et al. 2003).

Bayesian inference of the three datasets was performed using MrBayes 3.1 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). Likelihood parameters for Bayesian analyses were calculated using MrModeltest 2.2 (Nylander 2004). MrModeltest is a modified version of Modeltest 3.6 (Posada and Crandall 1998). It compares 24 instead of 56 models of nucelotide substitutions, performing hierarchical likelihood ratio test (hLRT)

and calculating approximate Akaike Information Criterion (AIC). GTR-I-Γ (for ITS) and GTR-Γ models (for ETS data matrix) were chosen under AIC and hLRT criteria. The models included six substitution rates; rates followed gamma distribution with four categories (ngamma=4) and sites were assumed invariable (for ITS) or variable (for ETS data set). Specific nucleotide frequencies were estimated from the data. In the combined data set, the overall rate was allowed to vary under a flat Dirichlet prior (priors were assumed unlinked between data partitions, allowing them to vary independently).

By default MrBayes uses Metropolis coupling to enhance the tree-climbing capabilities of the Markov chains. The method generates incrementally heated Markov chains. The heating flattens the posterior probabilities, thus allowing the chains to move through space more rapidly and find the isolated peaks in the posterior distribution and help the cold chain to avoid being trapped in the local optima (Huelsenbeck and Ronquist 2001, Ronquist et al. 2005). Two independent runs, each starting from independently generated random tree, were done. Both runs consisted of three incrementally heated and one cold chain. Each Markov chain was run for $2x10^6$ generations, and sampling was done each 100^{th} or 200^{th} cycle. To confirm that stationarity had been reached, the log-likelihoods of the chains were monitored graphically by plotting them against the generations for each run. The analyses were stopped once the average standard deviation of split frequencies between the two runs dropped below 0.01 (Ronquist et al. 2005). 25% of the trees were discarded in burn-in. The resulting 50% majority rule consensus tree was then imported and observed in TreeView (Page 1996)

Good bootstrap support (BS) was considered to be ≥70%, moderate support <70% and >50% and poor or no support BS ≤50% (Hills and Bull 1993). For Bayesian clade support

estimates good support was considered for posterior probabilities (PP) ≥0.90, moderate between PP <0.90 and PP >0.70, low to no support for PP ≤0.70 (Erixon et al. 2003, Randle et al. 2005, Werner et al. 2005).

Constraint Analyses. Constraint analyses testing monophyly of the subtribe Baccharidinae and a single origin of dioecy were conducted on the combined data set. Two files were created in MacClade v. 4.08, one in which *Baccharis, Archibaccharis* and *Heterothalamus* were forced to be monophyletic, and the other one in which *Aztecaster* was monophyletic with Baccharidinae. Both constraints were subjected to heuristic searches with parsimony optimality using Paup* 4.0b10. Recovered most parsimonious trees were used to construct the strict consensus tree, which was then compared with non-constrained strict-consensus of the most parsimonious trees using Shimodaira-Hasegawa test (Shimodaira and Hasegawa 1999) under full optimization option and bootstrap sampling of 1000 replications. The tree topologies were assumed to be significantly different if the $p<0.05$.

RESULTS

ITS Sequence Data. The length of the ITS region varied from 575 bp in *Brachycome ciliaris* to 635 bp in *Eucephalus glabratus* and *Zyrphelis decumbens*. Variation in length within *Hinterhubera* group was lower, ranging from 630 bp to 633 bp. The ITS-1 region was 250-255 bp long, the length of 5.8S subunit was constant at 164 bp, and the length of ITS-2 varied from 209 to 217 bp. The aligned matrix of the entire ITS region (ITS-1, 5.8S subunit and ITS-2) was 655 bp long of which 307 (46.9%) characters were constant, 82 (12.5%) variable characters were parsimony uninformative, and 266 (40.6%) characters were parsimony informative. Alignment required insertion of some indels of 1-2 bp long; a deletion of 55 bp characterized sequences of *Brachycome*. The uncorrected pairwise distance between

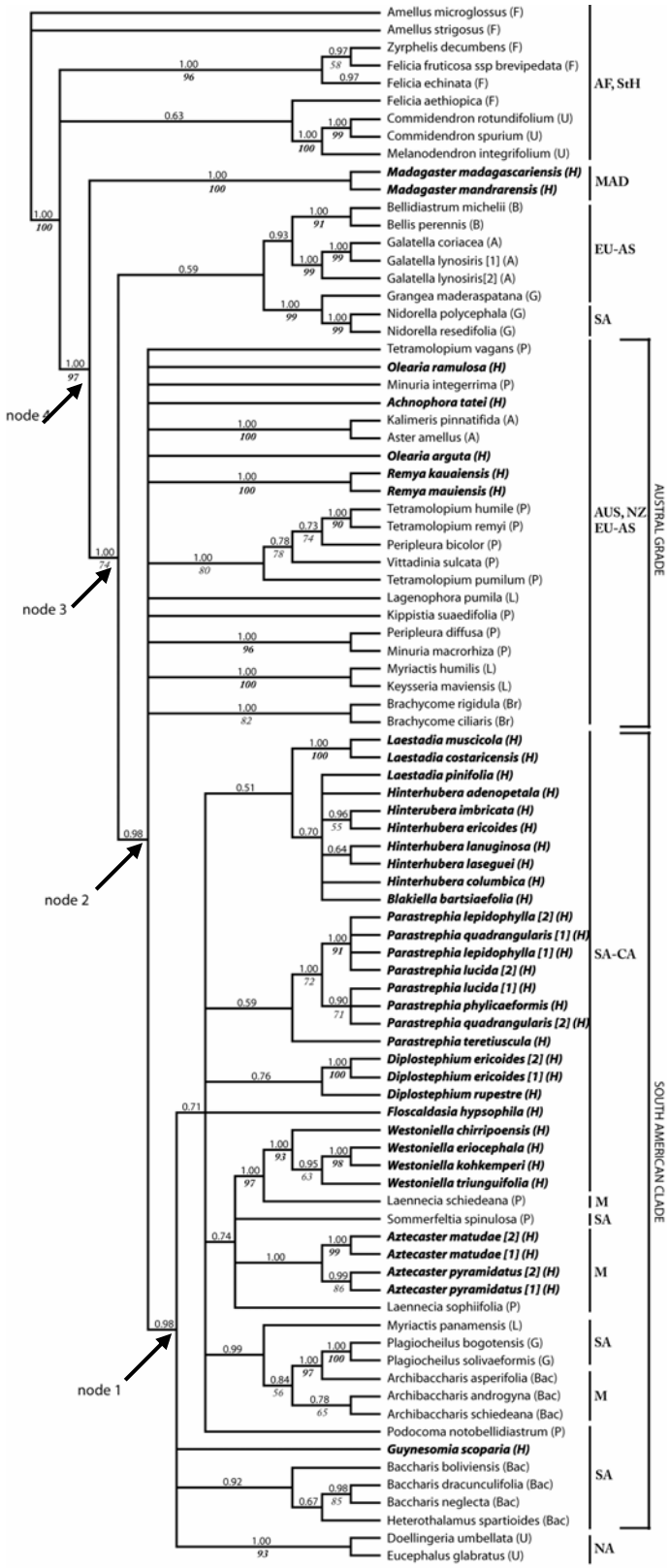
taxa ranged from 0% to 16%. The GC content varied from 49% to 54.5% and its mean was 52.83%.

MP analyses of the ITS data resulted in 2844 most parsimonious trees each 1270 steps long (CI=0.430, RI=0.588, RC=0.253). Bayesian analyses were run for $2x10^6$ generations, sampling each 200^{th} cycle. Resulting lnL values of the remaining trees ranged from -7651.33 to -7706.28. The 50% majority rule consensus tree based on Bayesian inference of ITS data is shown in figure 2.1.

ITS Trees. Four well supported clades (labeled at the nodes) were recovered on the ITS tree. The ITS data did not support the monophyly of the *Hinterhubera* group or of the other Southern Hemisphere subtribes: Grangeinae, Lagenophorinae, Podocominae and Baccharidinae. Asterinae also appeared polyphyletic, with *Galatella* Cass., *Bellis* L. and *Bellidiastrum* Less. being more closely related to Grangeinae (node 3, fig. 2.1) than to *Kalimeris* Cass. and *Aster amellus* (node 2, fig. 2.1).

Monophyly of the genera from the *Hinterhubera* group was supported in all analyses, with the exception of the genus *Laestadia*. Two species, *Laestadia costaricensis* and *L. muscicola* formed a well supported clade (PP = 1.00), while *L. pinifolia* was in a weakly supported (PP=0.54, BS<50%) clade with *Hinterhubera* and *Blakiella*. *Westoniella-Laennacia* clade was recovered in the analyses of the ITS data (BS=93%, PP=1.00), but their grouping with dioecious genus *Aztecaster* (PP=0.74) recovered in Bayesian analyses, was not supported in the parsimony analyses (BS<50%). Interspecific relationships within *Parastrephia* were not resolved. *Parastrephia teretiuscula* was sister to the other four species of the genus on the Bayesian inferred topology (PP=0.59), while such relationship received no

Figure 2.1 Phylogram inferred from Bayesian analyses and strict consensus tree of 2844 most parsimonious trees based on the ITS sequence data. Hinterhuberinae are in bold. Decimal numbers above branches are posterior probability values. Bootstrap support values (<50% not shown) are given below branches; values >90% are in bold. Species distribution is presented by vertical lines: AF=Africa; AUS=Australia; EU-AS=Europe and Asia; M=Mexico; MAD=Madagascar; NZ=New Zealand; SA-CA=South and Central America; StH=St. Helena. Subtribe names are abbreviated as follow: A=Asterinae; B=Bellidinae; Bac=Baccharidinae; Br=Brachycominae; F=Felicinae; G=Grangeinae; H=Hinterhuberinae; L=Lagenophorinae; NA=North American Astereae; P=Podocominae; U=unplaced.


Amellus microglossus (F)
Amellus strigosus (F)
Zyrphelis decumbens (F)
Felicia fruticosa ssp brevipedata (F)
Felicia echinata (F)
Felicia aethiopica (F)
Commidendron rotundifolium (U)
Commidendron spurium (U)
Melanodendron integrifolium (U)
AF, StH
Madagaster madagascariensis (H)
Madagaster mandrarensis (H)
MAD
Bellidiastrum michelii (B)
Bellis perennis (B)
Galatella coriacea (A)
Galatella lynosiris [1] (A)
Galatella lynosiris[2] (A)
Grangea maderaspatana (G)
EU-AS
Nidorella polycephala (G)
Nidorella resedifolia (G)
SA
Tetramolopium vagans (P)
Olearia ramulosa (H)
Minuria integerrima (P)
Achnophora tatei (H)
Kalimeris pinnatifida (A)
Aster amellus (A)
Olearia arguta (H)
Remya kauaiensis (H)
Remya mauiensis (H)
Tetramolopium humile (P)
Tetramolopium remyi (P)
AUS, NZ
EU-AS
Peripleura bicolor (P)
Vittadinia sulcata (P)
Tetramolopium pumilum (P)
Lagenophora pumila (L)
Kippistia suaedifolia (P)
Peripleura diffusa (P)
Minuria macrorhiza (P)
Myriactis humilis (L)
Keysseria maviensis (L)
Brachycome rigidula (Br)
Brachycome ciliaris (Br)
AUSTRAL GRADE
Laestadia muscicola (H)
Laestadia costaricensis (H)
Laestadia pinifolia (H)
Hinterhubera adenopetala (H)
Hinterubera imbricata (H)
Hinterhubera ericoides (H)
Hinterhubera lanuginosa (H)
Hinterhubera laseguei (H)
Hinterhubera columbica (H)
Blakiella bartsiaefolia (H)
Parastrephia lepidophylla [2] (H)
Parastrephia quadrangularis [1] (H)
Parastrephia lepidophylla [1] (H)
Parastrephia lucida [2] (H)
Parastrephia lucida [1] (H)
Parastrephia phylicaeformis (H)
Parastrephia quadrangularis [2] (H)
Parastrephia teretiuscula (H)
SA-CA
Diplostephium ericoides [2] (H)
Diplostephium ericoides [1] (H)
Diplostephium rupestre (H)
Floscaldasia hypsophila (H)
Westoniella chirripoensis (H)
Westoniella eriocephala (H)
Westoniella kohkemperi (H)
Westoniella triunguifolia (H)
Laennecia schiedeana (P)
M
Sommerfeltia spinulosa (P)
SA
Aztecaster matudae [2] (H)
Aztecaster matudae [1] (H)
Aztecaster pyramidatus [2] (H)
Aztecaster pyramidatus [1] (H)
M
Laennecia sophiifolia (P)
Myriactis panamensis (L)
Plagiocheilus bogotensis (G)
Plagiocheilus solivaeformis (G)
SA
Archibaccharis asperifolia (Bac)
Archibaccharis androgyna (Bac)
Archibaccharis schiedeana (Bac)
M
Podocoma notobellidiastrum (P)
Guynesomia scoparia (H)
Baccharis boliviensis (Bac)
Baccharis dracunculifolia (Bac)
Baccharis neglecta (Bac)
Heterothalamus spartioides (Bac)
SA
Doellingeria umbellata (U)
Eucephalus glabratus (U)
NA
SOUTH AMERICAN CLADE
node 1
node 2
node 3
node 4

support in the parsimony analyses. The two accessions of *Parastrephia quadrangularis* were not monophyletic. One grouped with *P. lepidophylla* and one accession of *P. lucida*, and the second one was in the sister clade clustered with *P. phylicaeformis* and the second accession of *P. lucida.* The two clades received moderate to high support (BS>69, PP=1.00). *Diplostephium* and *Guynesomia,* both genera from *Chiliotrichum* group, had unresolved positions within the South American clade. *Archibaccharis,* subtribe Baccharidinae, was sister to *Plagiocheilus,* subtribe Grangeinae, in a well supported clade (PP=1.00 and BS=97%) and appeared paraphyletic to *Baccharis* and *Heterothalamus* (Baccharidinae). South, Central and North American accessions of Hinterhuberinae (*Hinterhubera* group, *Diplostephium* and *Guynesomia*), Lagenophorinae, Podocominae, Grangeinae and Baccharidinae were grouped in a terminal well-supported (PP=0.98) but unresolved clade (node 1, fig. 2.1). In MrBayes inferred topology a polytomy, Austral grade, arose basal to this clade and was comprised of the subtribes Lagenophorinae, Podocominae, Hinterhuberinae and Asterinae represented by species from Australia, New Zealand, Hawaii, and Eurasia. The Madagascan genus *Madagaster* was basal to the well-supported (PP=1.00, BS=97%) ingroup taxa and only distantly associated with other Hinterhuberinae lineages (node 4, fig. 2.1).

ETS Sequence Data. Because primer regions were not well-conserved, various primer pairs at different loci were used for amplification and sequencing of the 3'ETS resulting in sequences varying in length. In order to reduce the amount of missing data, 120 characters were removed from the 5' end of the original matrix. The length of these truncated sequences varied from 441 bp in *Zyrphelis decumbens* to 452 in *Blakiella bartsiaefolia*, *Felicia aethiopica* and *Archibaccharis androgyna*. The final aligned matrix of the 3' ETS region was 475 bp long and it consisted of 210 (44.2%) constant characters, 92 (19.4%)

parsimony uninformative, and 173 (36.4%) parsimony informative characters. The length of the indels varied from 1-4 bp. The uncorrected pairwise distance between taxa ranged from 0% to 17.8%. The overall GC content varied from 44.9% to 52% and the mean was 49.1%. MP analyses of the ETS data resulted in 258,829 most parsimonious trees each 754 steps long (CI=0.508, RI=0.600, RC=0.305). Bayesian analyses were run for $2x10^6$ generations sampling each 200^{th} cycle. Resulting lnL values of the remaining trees ranged from -4766.65 to -4844.31.

ETS Trees. The tree topologies recovered by ETS data were congruent with the ITS topologies in the area of trees that had high clade support (fig. 2.2). Similarly to the ITS analyses, monophyly of the *Hinterhubera* group was not recovered by ETS data, while the monophyly of the genera from the group, with the exception of *Laestadia* was confirmed. *Laestadia costaricensis* and *L. muscicola* were in a well supported clade (PP=1.00, BS=96%) while the relationship of *L. pinifolia* was not resolved. Relationships within *Parastrephia* were unresolved. *Parastrephia teretiuscula* appeared basal to the other species (PP=0.96, BS<50%), but the relationship between the remaining four species showed the same pattern as in the ITS inferred phylogenies. *Westoniella-Laennecia* and *Archibaccharis-Plagiocheilus* clades were recovered with a high support (PP=1.00, BS=100% and PP=0.84, but BS<50%). Madagascan species of Hinterhuberinae were in the basal position relative to the remaining ingroup (PP=1.00, BS=100%).

Unlike the ITS data, the analyses of ETS dataset did not recover the South American clade and the Austral grade (node 1 and 2, fig. 2.1). Instead, all the genera, including the Asterinae-Grangeinae clade that was basal to the rest of the ingroup on the ITS topology, comprised an unresolved clade (node 1, fig. 2.2). There are several possible explanations for

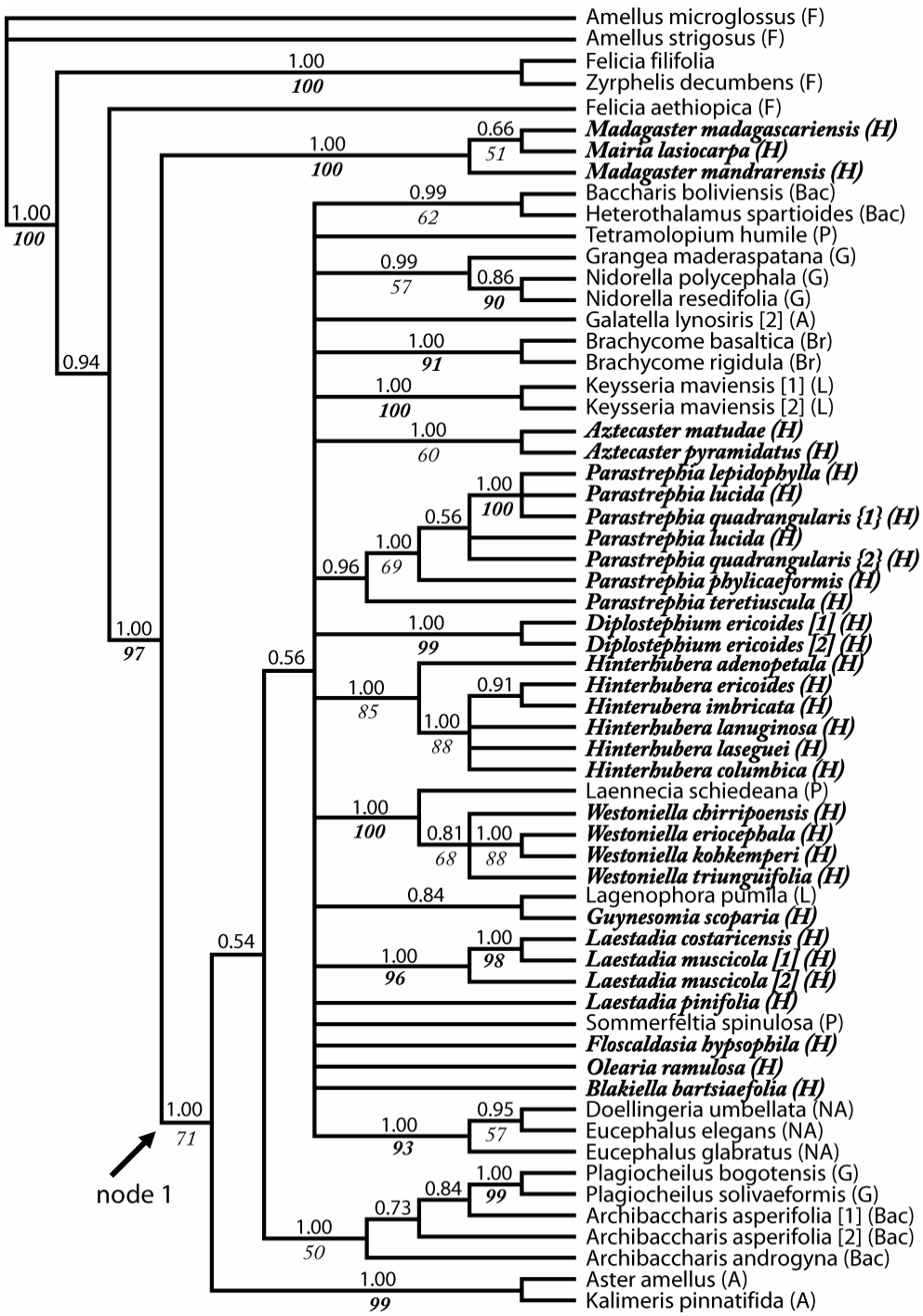


Figure 2.2. Phylogram inferred from Bayesian analyses and strict consensus of 258,829 most parsimonious trees based on ETS sequence data. Decimal numbers above branches are posterior probabilities for clades. Bootstrap support values (<50% not shown) are given below branches; values >90% are in bold. Subtribe abbreviations are as in figure 2.1.

this low resolution of the phylogenies based on the ETS dataset. One is the reduced sampling of genera in the ETS dataset. To test this option new and reduced ITS dataset that matched the ETS dataset in the taxa, was created and analyzed. Resulting tree topologies obtained by Bayesian inference and parsimony analyses were, however, fully congruent with those inferred from the complete ITS dataset, thus rejecting this option (data not shown). The other possibility is the low level of parsimony informative characters. In the ETS datamatrix it was lower (36%) compared to the ITS (40.6%), and as such, it explains the low resolution of the tree topologies based on the ETS dataset.

Combined Data. Because the ITS and ETS datasets could not be exactly matched for all the taxa, the combined ETS/ITS datamatrix included a reduced number of taxa. The aligned combined matrix was 1115 bp long of which 564 (50.6%) characters were constant, 197 (17.7%) variable characters were parsimony-uninformative and 354 (31.7%) characters were parsimony informative. The partition homogeneity test (Farris et al. 1994) confirmed that the two datasets were congruent (p=0.159).

Heuristic search using parsimony optimality criterion resulted in 659 most parsimonious trees each 1498 steps long, from two tree islands (CI=0.526, RI=0.579, RC=0.305). Bayesian analyses were run for $2x10^6$ generations sampling each 100^{th}. The lnL of the resulting trees varied from -9348.08 to -9379.44.

The tree topology recovered by parsimony and Bayesian analyses of the combined data sets (fig. 2.3) was highly congruent with the topologies inferred from the ITS data. Combining data resulted in increased support for certain clades. Support for the *Aztecaster-Westoniella* clade increased from PP=0.74 to PP=0.87; support for the clade between *Parastrephia teretiuscula* and the other four *Parastrephia* species increased from PP=0.59

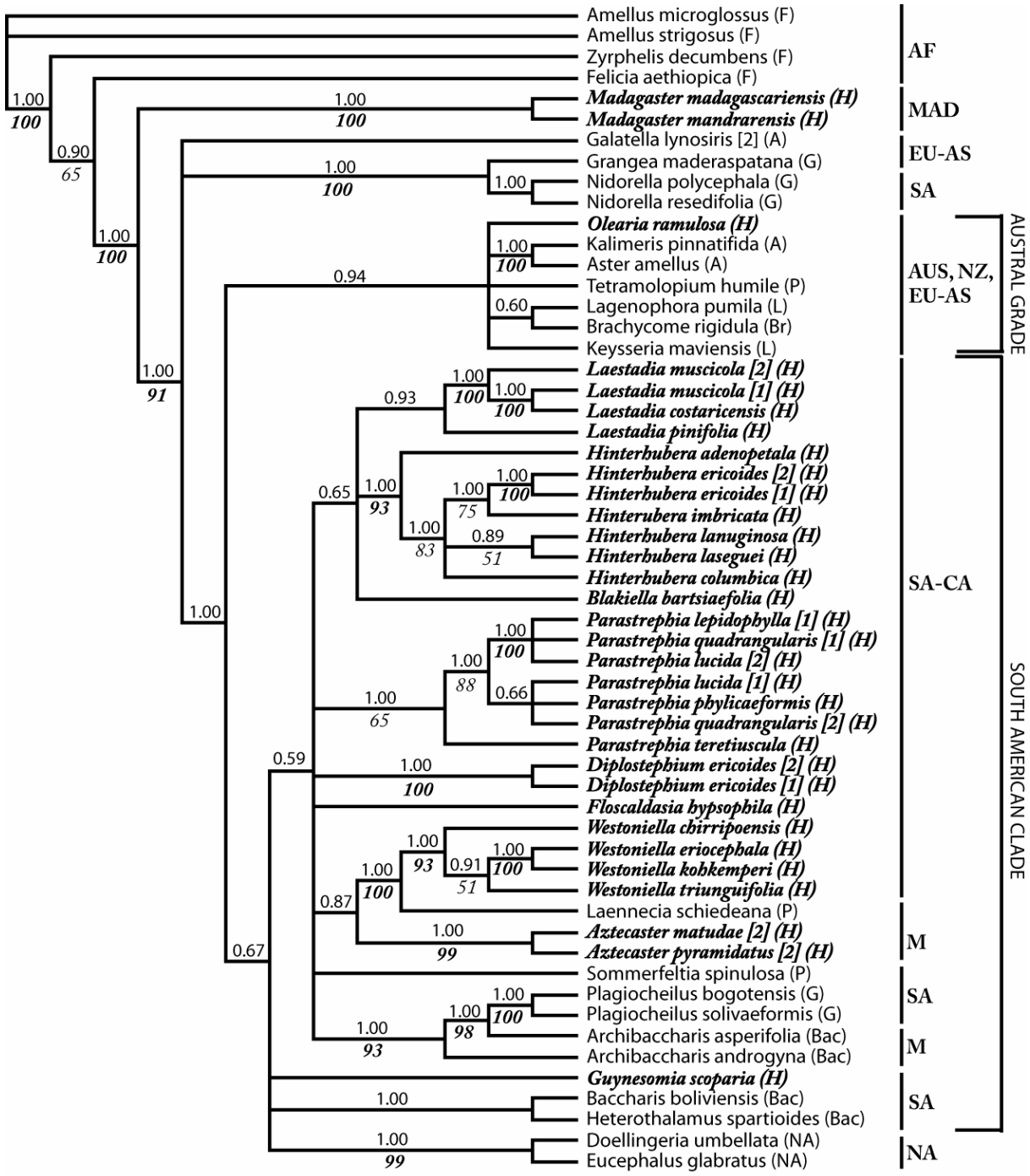


Figure 2.3. Phylogram inferred from Bayesian analyses and strict consensus tree of 659 most parsimonious trees based on the combined sequence data. Decimal numbers above branches are posterior probability values. Bootstrap support values (<50% not shown) are given below branches; values >90% are in bold. Subtribe abbreviations and species distribution are as in figure 2.1.

and BS<50% to PP=1.00 and BS=65%. Support for *Hinterhubera-Laestadia-Blakiella* clade (PP=0.65, BS<50%) provided low justification to consider those genera as close relatives. Unlike the ITS and ETS data sets, the combined analyses recovered the monophyletic *Laestadia* with high support (PP=0.93, but BS<50%).

Constraint Analyses. MP analyses of the combined data that forced monophyletic *Aztecaster* and Baccharidinae resulted in trees 1597 steps long, 99 steps longer than the unconstrained topologies (data not shown). The strict consensus tree inferred from the constraint dataset was significantly worse (p=0.01) than the one based on the unconstraint analyses as inferred by the Shimodaira-Hasegawa test. The constraint analyses that forced monophyletic Baccharidinae resulted in trees 1501 steps long, 3 step longer than the unconstrained topologies.

DISCUSSION

South American Clade and Austral Grade. Representatives of Asterinae, Lagenophorinae, Podocominae, Brachycominae, Baccharidinae and Hinterhuberinae were grouped in a partially resolved South American clade (SA) and Austral grade. The former was sister to the North American clade (NA) represented by *Doellingeria* and *Eucephalus* (figs. 2.1, 2.3). Basal to the SA and Austral grade were Grangeinae, Asterinae, Bellidinae and *Madagaster* (Hinterhuberinae) (nodes 3 and 4, fig. 2.1). The Bayesian inferred topology of ITS and combined datasets recovered *Hinterhubera* group within the SA clade as part of a polytomy with representatives of Lagenophorinae, Podocominae, Baccharidinae and two other genera from Hinterhuberinae, *Diplostephium* and *Guynesomia* (fig. 2.1). Sister to this clade was Austral grade that formed a polytomy containing representatives of Hinterhuberinae, Lagenophorinae, Asterinae, Podocominae and Brachycominae. Subtribes

Asterinae, Lagenophorinae, Podocominae and Hinterhuberinae are clearly non-monopyletic based on the results of the present study.

Mapping of the geographic areas occupied by the species on the Bayesian inferred phylogeny revealed an unexpected pattern of relationship. Clade SA included only those representatives of Hinterhuberinae, Baccharidinae, Podocominae and Lagenophorinae that occur in South and Central America or Mexico (figs. 2.1, 2.3). The Austral grade that formed a polytomy basal to the SA clade included taxa assigned to the same subtribes but restricted to Australia, New Zealand, New Guinea, Hawaii, Africa and Eurasia. These phylogenies suggested a closer relationship among taxa from similar geographic areas rather than according to their existing subtribal assignments. One note of caution is that this study focused on phylogeny of Hinterhuberinae, and sampling within other subtribes distributed in Southern Hemisphere was limited. Further sampling of these subtribes and addition of unlinked DNA data such as chloroplast DNA, are needed to further test the relationship among the Southern Hemisphere subtribes. Based on analyses of ITS and ETS data, morphological characters presently used to delimit the subtribes appear to be homoplastic.

***Hinterhubera* Group.** Both ITS and ETS failed to support the monophyly of the *Hinterhubera* group. Instead they were placed in a clade with Baccharidinae, some genera being more closely related to other subtribes than the Hinterhuberinae itself (figs. 2.1-2.3). The monophyly of genera in the *Hinterhubera* group except for *Laestadia* was supported. The hypothesized close relationship of *L. muscicola* and *L. costaricensis* (Cuatrecasas 1968) was well supported with PP=1.00 and BS>98% in all topologies. The two species are morphologically similar in their creeping habit and leaves loosely arranged along the stem, pubescent stem, disk florets with trichomes on tubes and limbs, and shortly beaked cypselae.

Laestadia pinifolia was either in a clade basal to these two species (BS<50%, PP=0.93; fig. 2.3) or was unresolved within the SA clade - Austral grade (node 1, fig. 2.2). Unlike the previous two species, the leaves in *L. pinifolia* are densely arranged along the stem, the stem is glabrous, disk florets lack trichomes on tubes and limbs, and cypselae have well defined beak. Because of the epappose, beaked cypselae, *Laestadia* was thought to be related to *Lagenophora*, *Solenogyne*, *Myriactis*, and *Keysseria* (Cabrera 1966) a hypothesis not supported by molecular analyses. Instead, Australasian *Myriactis* and Hawaiian genus *Keysseria* clustered together in a well supported clade and together with the New Zealand species *Lagenophora pumila* were part of the unresolved Austral grade, distant from South American *Laestadia* (figs. 2.1, 2.3). Similarly, the hypothesized relationship with Grangeinae (Robinson and Bretell 1973) and Bellidinae (Cuatrecasas 1986) was not confirmed. Similarity of the cypselae of Grangeinae and *Laestadia* (presence of beak and absence of pappus) is indeed due to convergent evolution, as suggested by Nesom (1993b, 1994a). The multinerved, partially compressed cypselae and disk florets with sterile ovaries nest *Laestadia* in Hinterhuberinae. Habitually, *Laestadia* resembles *Floscaldasia* and both are considered among the most specialized members of the group (Nesom 1994a, Nesom 2000). Unlike *Laestadia*, *Floscaldasia*'s cypselae are not beaked, they bear pappus, and minute, about 1 mm long ray florets have well developed ligules. Despite morphological similarity, close relationship between *Laestadia* and *Floscaldasia* was not supported in the present molecular phylogenies. Instead, analyses of the ITS and the combined data resolved *Laestadia* sister to *Blakiella* and *Hinterhubera*, although support was weak (PP≤0.65 and BS≤50%; figs. 2.1, 2.3) and not recovered by ETS data. *Blakiella* is the only other genus in the subtribe Hinterhuberinae with beaked cypselae. Also, its cypselae are glabrous, 4-nerved and have a

pappus of scabrid bristles (5-8 nerved and epappose in *Laestadia*). Reduction in pappus is not unusual among closely related genera. It is found for example in the subtribes Felicinae and Lagenophorinae, and loss of pappus is known within genera (i.e. *Boltonia*, *Calotis*, *Podolepis* and others; Fernald 1940, Nesom 2000, Koshini et al. 2000). An immediate relationship between *Hinterhubera* and *Laestadia* is not apparent. Species of *Hinterhubera* are small shrubs with coriaceous leaves, while *Laestadia* are prostrate perennial herbaceous plants or subshrubs with leaves either coriaceous (in *L. pinifolia* and *L. rupestris* Benth.) or more or less herbaceous (in *L. muscicola*, *L. lechlerii* Wedd. and *L. costaricensis*). Cypselae in *Hinterhubera* are sericeous, pappose and 2-3(5) nerved versus glabrous, epappose, and multinerved (5-8) in *Laestadia*. However, the two genera have similar, tubular ray florets that differ in size and ligulate lobbing. In *Hinterhubera* the lobes are long and irregular while in *Laestadia* the ray florets are minute with short regular lobes.

Floscaldasia hypsophila had an unresolved position within the *Hinterhubera* group, and its pervious placements within Asterinae (Cuatrecasas 1969), Grangeinae (Robinson and Brettell 1973), Bellidinae (Cuatrecasas 1986) or *Lagenophora* group of the subtribe Asterinae (Bremer 1994) was not corroborated. Although its position in the SA clade was unresolved, its affinities appear to be with other highly specialized elements of the subtribe, *Laestadia* and *Westoniella* (Nesom 1994a, Nesom 2000).

Westoniella consists of two distinct morphotypes based on the habit and capitulescence, small, dwarf shrubs with solitary capitula, and tall shrubs with capitula in terminal corymboid, glomerate panicles. Each morphotype was presented with two species in the analyses, dwarf shrubby *W. chirripoensis* and *W. triunguifolia* and tall shrubby *W. eriocephala* and *W. kohkemperi*. Present analyses supported the tall shrubby species as

derived (fig. 2.1). Cuatrecasas (1977) included the genus *Westoniella* in Asterinae because of its unusually reduced ray florets that he assumed to be of primitive *Aster* type. Present molecular phylogenies did not support such a relationship, but were in agreement with its later transfer to Hinterhuberinae (Cuatrecasas 1986). The genera that resemble *Westoniella* in habit and other features are the Andean *Blakiella*, *Hinterhubera* and *Diplostephium* (Cuatrecasas 1977). *Hinterhubera* differs in deeply lobed ray florets and in the presence of several series of pappus bristles. *Blakiella* has bottle-shaped ray florets with short, but defined ligule and, unlike *Westoniella*, its cypselae are beaked. *Diplostephium* differs from *Westoniella* in having ligulate ray florets and biseriate pappus bristles. Neither of the phylogenies provided support for the close affinity of *Westoniella* and mentioned genera, nor was the postulated close affinity with *Flosmutisia*, *Floscaldasia* and *Laestadia* (Nesom1993b) confirmed. Instead, all these genera were part of the unresolved SA clade (figs. 2.1, 2.3).

Unexpectedly, *Westoniella* formed a strongly supported (BS=98%, PP=1.00; figs. 2.1-2.3) clade with *Laennecia schiediana.* An accession of *Laennecia sophiifolia* obtained from GenBank was sister to *Westoniella-Laennecia* clade, although with a weak support (PP=0.52, fig. 2.1). *Laennecia* is a genus currently placed in the subtribe Podocominae (Nesom 1994a, Nesom and Robinson 2006). It is distributed in montane habitats or temperate highlands of South America (Peru, Argentina, Bolivia, Venezuela, Ecuador, and Colombia) extending north over Central America and Mexico into southwestern United States. Features shared by *Westoniella* and *Laennecia* include epaleate receptacle and minute ray florets with reduced ligule, compressed, glabrous cypselae with two marginal ribs and a pappus of barbellate bristles. Densely tomentose leaves and phyllaries typical in *Westoniella* are also present in *L. schiediana* and other species of the sect. *Laennecia*. Multicellular type of trichomes found on

the phyllaries in *W. eriocephala* and *W. kohkemperi* (also present in *W. lanuginosa* Cuatr. and *W. barqueroana* Cuatr. two species not included in the analyses), are found on the phyllaries of *L. schiediana* and some other species of *Laennecia* (Nesom 1990). Unlike *Westoniella*, disk florets in *Laennecia* are fertile.

Hypothesized close relationship between *Parastrephia* and *Lepidophyllum* (Bentham and Hooker 1873, Hoffman 1890, Cabrera 1945, Nesom 1993c) was not supported with ITS and ETS data. *Lepidophyllum* as part of the basal group of Hinterhuberinae (not presented here, but see chapter 3, 4) is characterized by opposite leaves, radiate capitula and flattened pappus bristles. *Parastrephia*, however, with discoid capitula, ray florets with much reduced ligules and scabrid, not flattened pappus bristles is well nested within the *Hinterhubera* group (figs. 2.1, 2.3).

The interspecific relationships in *Parastrephia* remained unresolved. The genus initially included five species (Cabrera 1954), later it was reduced to three (Nesom 1993c). The analyses did not confirm either of the taxonomic treatments of the genus. *Parastrephia teretiuscula* had basal position within the genus (PP=0.59-1.00, BS≤50%, figs. 2.1-2.3). The species is easily recognized and distinguished from the others by densely arranged leaves apressed against the stem (and somewhat resembling *Lepidophyllum* leaves). The remaining four species (*sensu* Cabrera 1954) were grouped in two sister clades, each containing accessions of *P. lucida* and *P. quadrangularis*, and either *P. lepidophylla* or *P. phylaceiformis*. There are two main possible explanations for the multiple terminals of the same species, i.e. *P. lucida* and *P. quadrangularis*. One is lineage sorting which is likely to be seen near the species level (Maddison 1997). The addition of other unlinked DNA sequences and increased population sampling is necessary to test this hypothesis. Another possibility is

that the interspecific hybridization has resulted in reticulate evolution. The geographical distribution of the two species groups would allow for hybridization to occur. Current data do not allow for distinction between these two processes.

Phylogenetic relationships of *Flosmutisia* were not investigated. An affinity to *Hinterhubera* is hypothesized due to morphological similarity of their ray florets. Both genera possess tubular, zygomorphic corollas with five irregular lobes.

Guynesomia* and *Diplostephium. Present study corroborated the recent segregation of *Guynesomia* from *Nardophyllum* (Bonifancino & Sancho 2004). *Guynesomia scoparia* occupied basal position to *Hinterhubera* group (fig. 2.1, 2.3) or was unresolved in the large polytomy (node 1, fig. 2.2). No relation to *Nardophyllum* (from the *Chiliotrichum* group *sensu* Nesom 1994a) was recovered (data not shown, see chapter 4). Unlike other *Nardophyllum* species, *Guynesomia* has disciform, heterogamous capitula, epaleate receptacle and numerous capitula that are arranged in panicle-like or raceme-like capitulescence. *Nardophyllum* with discoid, homogamous, solitary capitula and paleate receptacle nests within *Chiliotrichum* group. Previous treatment of *G. scoparia* within *Hinterhubera* (Cabrera 1954) was rejected by molecular analyses, and could not be supported by morphology either. Disk florets in *Hinterhubera* are functionally staminate, and ray corollas are tubular, deeply 4-5-lobed, zygomorphic to actinomorphic, unlike the bisexual disk florets and clearly zygomorphic ligulate ray corollas in *Guynesomia*. Postulated close phyletic relationship of *Guynesomia* and the radiate *Diplostephium* (Bonifancino and Sancho 2004) was not confirmed in present analyses.

Diplostephium is a large genus of ca. 90 species distributed in the páramos from Costa Rica to northern Chile. Nesom (1994a) placed it in the informal *Chiliotrichum* group of the

subtribe Hinterhuberinae based on its radiate capitula and partially paleate receptacles. Previous phylogenetic analyses recovered its derived and only distant relationship to the *Chiliotrichum* group (Noyes and Rieseberg 1999). Similar results were obtained in the present study. *Diplostephium* was placed within SA clade (fig. 2.1). The genus includes tall shrubs and small trees with radiate capitula, mostly epaleate receptacles, and staminate or sometimes bisexual disk florets. The most interesting feature in the morphology of *Diplostephium* is the stylar variation in disk florets. Three types are recognized based on the style branch length. In type one the styles are merely bifid or subentire, types two and three consist of medium length and elongate branches respectively. Blake (1922, 1928) hypothesized the second or the third type to be primitive and the other two derived. In either case, as suggested by Blake (1928), the evolution of the genus was directed towards development of two groups, one with subentire, slightly bifid styles and sterile disk florets and the other with elongated styles and fertile disk florets. Present analyses of the ITS data included two species, *D. rupestre* with the first style type and *D. ericoides* with the third style type. The two formed a monophyletic clade within the SA lineage. Phylogenetic placement of *Diplostephium* and inclusion of *Guynesomia scoparia* in the *Hinterhubera* group suggests that evolutionary tendency within Hinterhuberinae is the transition from radiate capitula with bisexual disk florets towards taxa with discoid and disciform capitula with staminate disk florets, and finally dioecy.

Evolution of Dioecy in Hinterhuberinae. Dioecy, as postulated by Nesom (1993a), had evolved independently in *Aztecaster* and in Baccharidinae. Phylogenetic analyses of the ITS and ETS data corroborated this hypothesis, resolving *Aztecaster* distantly related to *Archibaccharis* and *Baccharis-Heterothalamus* clades (fig. 2.1).

Within Hinterhuberinae, *Aztecaster* was sister to *Westoniella-Laennacia* clade with week support (PP<87, BS<50%). The fact that *Laennecia* and *Westoniella* have shortly ligulate to almost tubular ray corollas and staminate disk florets suggests that the pistillate capitula in *Aztecaster* may have evolved from discoid or possibly radiate capitula by complete reduction of ligules in marginal pistillate corollas and loss of staminate disk florets. Similar transitions in morphology of pistillate corollas from short ligulate (paralleling *Laennecia*) to truncate (similar to *Westoniella*) or five-dentate (like in *Aztecaster*) are present in *Baccharis* s.l. (Hellwig 1997, Guiliano 2001). The possibility that these three genera share a common ancestor is strengthened by the fact that they occur in geographical proximity, *Westoniella* is distributed in páramos of Costa Rica and adjacent Panama, *Aztecaster* in grasslands and matorals in southern and central Mexico, and *Laennecia schiediana* in hillsides and meadows at timberline in Mexico and southern North America.

Another way dioecy may have evolved in *Aztecaster* is suggested by the unusual pistillate florets present in *Archibaccharis penninsularis* Blake relative to other species in that genus. Its pistillate plants bear only homogamous capitula with tubular corollas with five regular lobes (similar in morphology to the ones in *Aztecaster*), and vestigial anthers in the tubes (Jackson 1975). Presence of vestigial anthers suggests that the pistillate corollas evolved from bisexual, tubular disk florets in a way similarly postulated for *Aztecaster* by Nesom (1993a). Throughout the genus *Archibaccharis* pistillate corollas are zygomorphic, hence, "retention of these aberrant pistillate florets with concurrent loss of all ray flowers from an ancestral heterogamous head may represent yet a different evolutionary experiment to achieve dioecism" (Jackson 1975).

In conclusion, molecular analyses suggest that different forms of pistillate ray corollas in Hinterhuberinae and Baccharidinae probably evolved independently of each other, as a result of parallel evolution. Further sampling of Baccharidinae will be needed to better understand the relationship of dioecy between these two subtribes.

Baccharidinae. Earlier molecular analyses of cpDNA recovered a monophyletic Baccharidinae (Zanowiak 1991). *Archibaccharis* and *Heterothalamus* formed a monophyletic clade that was basal to *Baccharis* and its segregates. Present analyses of ITS and ETS data resolved *Baccharis* and *Heterothalamus* in a clade basal to the polytomy containing *Archibaccharis* and the *Hinterhubera* group suggesting a polyphyletic Baccharidinae (fig. 2.2). Constraint analyses that forced monophyletic Baccharidinae resulted in topologies only one step longer than the unconstrained. This small increase in length indicates that there is no conflict in the taxonomic treatment of the subtribe and the molecular data.

Archibaccharis is subdioecious with a tendency for androdioecy (Hellwig 1996). As such the genus has been considered an intermediate step in the evolution of dioecy in Baccharidinae (Jackson 1975, Nesom 1993a). Functional androdioecy is extremely rare breeding system in which populations contain two sexes, males and hermaphrodites (Wolf et al. 1997, Pannell 2002, Wolf and Takebayashi 2004). It evolved probably from dioecious and less likely from hermaphroditic stage (Wolf and Takebayashi 2004). Functional androdioecy has been confirmed for small number of species (reviewed in Pannell 2002), while the other cases of androdioecy in fact refer to the morphological androdioecy, and are functionally dioecious, subdioecious or andromonoecious stages (Wolf et al. 1997). Because the *Archibaccharis* species lack fertile anthers in central disk florets of the hermaphroditic capitula, the androdioecious condition can be considered to be of morphological character.

The flower morphology of pistillate florets in species of *Archibaccharis* suggests that subdioecious condition in the genus probably evolved from hermaphroditic florets (Jackson 1975).

Unexpectedly, *Archibaccharis* grouped together with *Plagiocheilus*, a South American genus (PP>0.84, BS>93). The possibility of contamination of DNA was eliminated repeating the extraction of DNA from the two genera at different times. *Plagiocheilus* is placed in Grangeinae based on its herbaceous habit, pinnatifid leaves, short ligules barely exceeding the involucres, and lack of pappus (Nesom 1994a, Nesom and Robinson 2006). Its relationship with *Archibaccharis* recovered in present analyses is unclear. The two genera are morphologically very dissimilar. *Plagiocheilus* includes herbaceous monoecious perennials, with bilabiate ray corollas, while *Archibaccharis* are subdioecious shrubs and small trees. In addition, pistillate corollas in *Archibaccharis* vary in shape from shortly ligulate to truncate, but none of them exhibit bilabiate condition. The main difference is the presence of glochidiate trichomes on the cypselae of *Plaghiocheilus*. This type of trichomes is otherwise rare feature in the tribe Astereae and it is absent in Baccharidinae. The two genera are also geographically isolated. *Plagiocheilus* is restricted to the montane habitats from Colombia to Argentina, while *Archibaccharis* is primarily montane Mexican and Central American with only one species described from South America (Bolivia). This unusual lineage warrants further investigation.

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CHAPTER 3 PHYLOGENY OF *CHILIOTRICHUM*, *NOVENIA* AND RELATED GENERA (HINTERHUBERINAE: ASTEREAE)

Centered about *Chiliotrichum* are eleven genera generally characterized by a shrubby habit, resinous, usually white-tomentose leaves, radiate capitula, paleate receptacles and bisexual disk florets, and distributed mainly in the southern part of the Andes of South America with one genus reaching to Mexico (tab. 3.1). They form the informal *Chiliotrichum* group *sensu* Nesom (1994). Members of the group have been variously treated; *Nardophyllum* was placed within Mutisiae (Weddel 1857), *Llerasia,* as section Diplostephioides of *Haplopappus* Cass., and *Diplostephium* were regarded within Asterinae (Weddel 1857), *Diplostephium* and *Oritrophium* had been placed within Asterinae (Cuatrecasas 1969) and *Pteronia* and *Llerasia* in Solidagininae (Bremer 1994, Zhang and Bremer 1993, Cuatrecasas 1969). A close relationship between the seven genera, *Nardophyllum*, *Aylacophora*, *Parastrephia*, *Chiliotrichum*, *Lepidophyllum*, *Chiliophyllum* and *Chiliotrichiopsis,* was first observed by Cabrera (1954), although earlier Blake (1922) remarked the similarity of *Chiliotrichum* to *Diplostephium* and *Hinterhubera* Sch. Bip.. Because of homochromous capitula (yellow ray and disc flowers), and somewhat "*Solidago*-like" style appendages, characters observed in *Chiliophyllum*, *Chiliotrichum*, *Parastrephia*, *Chiliotrichiopsis*, *Nardophyllum* and *Aylacophora*, Nesom (1991) speculated that this group, in Cabrera's more restricted sense, belonged to the subtribe Solidagininae, although the nature of the relationship to the rest of the Solidagininae was unclear. Morphological-based cladistic analyses of the tribe Astereae by Zhang and Bremer (1993) resulted in the *Chiliotrichum* group, represented by the genus *Chiliotrichum*, nested well within subtribe Asterinae. They expanded the group to include *Diplostephium* and *Lepidophyllum*, while two other genera, *Llerasia* and *Pteronia,*

Table 3.1. Geographic distribution of the genera from the informal *Chiliotrichum* (C) and *Novenia* (N) groups.

Genus	# of species	Distribution
Aylacophora Cabrera (C)	1	Argentina
Chiliophyllum Phil. (C)	3	Argentina, Chile
Chiliotrichiopsis Cabrera (C)	4	Argentina, Peru
Chiliotrichum Cass. (C)	2	Argentina, Chile
Diplostephium H.B. & K. (C)	ca. 80	Chile, Peru, Bolivia, Ecuador, Colombia, Costa Rica, Panama
Lepidophyllum Cass. (C)	1	Patagonia
Llerasia Triana (C)	14	Bolivia, Peru, Ecuador, Colombia
Nardophyllum Hook & Arn. (C)	5	Argentina, Chile, Bolivia
Oritrophium (H.B. & K.) Cuatr. (C)	20	Mexico, Venezuela, Colombia, Bolivia, Ecuador, Peru
Paleaepappus Cabrera (C)	1	Patagonia
Pteronia L. (C)	ca. 80	southern Africa, Zimbabwe
Novenia S.E. Freire (N)	1	Peru, Bolivia, Argentina

were placed within subtribe Solidagininae, as part of the *Haplopappus* and *Engleria* groups respectively (Zhang and Bremer 1993, Bremer, 1994). Nesom (1993a, 1994) delimited the group and placed it in the redefined subtribe Hinterhuberinae. The recent ITS-based phylogeny of North American Astereae (Noyes and Rieseberg 1999) included *Oritrophium*, *Diplostephium*, *Chiliotrichum* and *Pteronia*. In their topology, the group failed the test for monophyly. *Diplostephium* was in the Southern Hemisphere grade, while the other three genera in their basal group as sister taxa to *Commidendrum* Burch. ex DC. and representatives of subtribe Felicinae. In the analyses of Cross et al. (2002) *Chiliotrichum* and *Oritrophium* were separated from each other, occupying basal positions in two well-defined primary

clades, with *Olearia* being distributed between these two clades. Similar results were confirmed in phylogenetic analyses of *Bellis* L. (Fiz et al 2002).

Somewhat isolated in the group is the genus *Oritrophium*. The genus is distributed in páramos of South America, with two species recently described from Mexico (Nesom 1992, 1998). *Oritrophium* and *Aztecaster* Nesom, the later from *Hinterhubera* group, are the only members of Hinterhuberinae reaching into North America. Because of its herbaceous habit, *Oritrophium* was hypothesized to be highly derived member of the subtribe (Nesom 1993a). The genus was first recognized as section *Oritrophium* of *Aster* L. (Humboldt et al. 1820) and later of *Erigeron* L. (Weddell 1857, Bentham and Hooker 1873). Its rosulate habit, scapose inflorescences, and staminate disk florets defined the new genus *Oritrophium* and suggested a possible close relationship to *Diplostephium* (Cuatrecasas 1961). Similarity of *Oritrophium* to the New Zealand genus *Celmisia* Cass. was pointed out by several authors (Scultz-Bipontinus 1856; Nesom 1992, 1993a, 1994), and consequently Solbrig (1960) transferred the section *Oritrophium* from *Erigeron* to *Celmisia*. Nevertheless, Cuatrecasas (1961, 1997) maintained its generic status distinct from *Celmisia*, which is characterized by large, stout rosulate plants, and always fertile, hermaphroditic disk florets. In addition, the chromosome numbers of the two genera differ greatly. *Celmisia* (x=9) is from hexaploid to dodecaploid, while *Oritrophium* (x=9) is diploid or tetraploid (Dawson 2000, Nesom 1992).

Novenia is a recently described monotypic genus from high elevations of the Peruvian Andes. It was considered in the subtribe Gnaphaliinae of Inuleae (Freire 1986; Freire and Hellwig 1990), it was related to the genera of the Inuleae *sensu stricto* (Anderberg 1989), or listed within genera with unknown position in the subfamily Asteroideae (Anderberg 1991, Bremer 1994). *Novenia* is highly reduced in size (1.5-3.5 cm in diameter) and specialized in

vegetative and floral morphology. Its shortly sagittate anthers, sericeous, compressed cypselae, pappus of barbellate bristles and its chromosome number (n=9) are typical of Astereae. *Novenia*'s placement in subtribe Hinterhuberinae is suggested by its rigid leaves with auxiliary tufts of tomentum, disciform capitula with reduced pistillate corollas, and functionally staminate disk florets, multinerved cypselae and multiseriate pappus of persistent bristles (Nesom 1994). However, further refinement of its relationship to other genera in the subtribe is difficult to assess due to its highly reduced size and unique features.

The objectives of this research were to 1) investigate the monophyly of the *Chiliotrichum* group with a wide sample of genera, 2) test the hypothesis of Cuatrecasas (1961, 1997), Solbrig (1960) and Nesom (1992, 1993a, 1994) regarding the relationship of *Oritrophium* and other genera within Hinterhuberinae; 3) assess the relationship of *Novenia* within the subtribe and 4) using the molecular estimates of phylogeny to evaluate morphological characters that were cited as indicators of generic-level relationships.

MATERIALS AND METHODS

Taxon Sampling. Sampling of the taxa was designed to include at least one species from the majority of the currently recognized genera from the Nesom's (1994) informal *Chiliotrichum* group. Nine out of 11 genera were sampled. Sampling also included the monotypic genus *Novenia*. The monotypic genera *Aylacophora* and *Paleapappus* were not included because of the lack of plant material. All extractions were done from leaf material removed from herbarium specimens, or plant material dried in silica gel crystals. Herbarium material was obtained from following herbaria: F, LSU, MERC, MO, NO, NY, QCA, QCNE, SGO, TEX/LL, US, and USM, (acronyms follow the Index Herbariorum, New York Botanical Garden, http://sciweb.nybg.org/science2/IndexHerbariorum.asp). Silica dried

material was kindly provided by M. Bonifancino (Facultad de Agronomia, Laboratorio de Botanica, Montevideo, Uruguay). Identification of the species was confirmed using keys published in regional floras (Black 1957, Allan 1961, Aristeguieta 1964, Koster 1966, Cuatrecasas 1969, Cabrera 1971, Stanley and Ross 1983, Wagner et al. 1990, Herman et al. 2000) and other taxonomic works (Hutchinson and Philips 1917, Cabrera 1945, 1954, Humbert 1960, Cuatrecasas 1970, 1997, Nesom 1992, 1993b, Nesom et al. 2001, Bonifancino and Sancho 2001, 2004).

The choice of the genera included in the dataset was based on the previous morphological (Drury 1968, Given 1973, Cuatrecasas 1986, 1997, Zhang and Bremer 1993, Nesom 1994, Nesom et al. 2001) and molecular analyses (Noyes and Rieseberg 1999, Lowrey et al. 2001, Cross et al. 2002, Fiz et al. 2002, Eastwood et al. 2004). To assess the relationship of these genera within the tribe Astereae, one to three genera from Southern Hemisphere subtribes, Felicinae and Grangeinae, were included in the dataset. In addition representatives from Asterinae and Bellidinae, genera with primarily Old World distribution were sampled. *Commidendrum* and *Melanodendron* DC. were represented only in the ITS data set each with one accession. Two genera, *Doellingeria* Nees. and *Eucephalus* Nutt., were chosen as representatives of the North American clade *sensu* Noyes and Rieseberg (1999) because of their basal position in this lineage. The selection of genera from the rest of the Hinterhuberinae included in the present study was based on the molecular analyses presented in chapter 4. Consequently representatives of *Celmisia*, *Olearia*, and *Madagaster* groups were included. *Diplostephium* and *Hinterhubera* group (*sensu* Nesom 1994) were not included because initial analyses (see chapters 2 and 4) did not show any correlation between the two

groups. Relationship of *Chiliotrichum* and *Hinterhubera* group (*sensu* Nesom 1994) is discussed in more details in chapter 4.

The morphological analyses of Astereae (Zhang and Bremer 1993) resolved *Grangea* Adans. as sister to the rest of the tribe. In the analyses of chloroplast DNA data, the restriction site (Jansen et al. 1990) and the ndhF sequence variation (Jansen and Kim 1994), *Felicia bergeriana* O.Hoffm. ex Zahlbr. was sister taxon to other representatives of the tribe Astereae, though both analyses included limited sampling. Analyses based on nuclear ribosomal DNA data (Noyes and Rieseberg 1999) were rooted using representatives of tribes Anthemideae, Calenduleae, Gnaphalieae and Inuleae. In those analyses *Grangea* was embedded in Astereae, while Felicinae were basal and sister to the *Chiliotrichum* group. The analyses of ITS sequence data of Cross et al. (2002) rooted with Anthemideae recovered *Chiliotrichum* and part of *Olearia* in a basal clade, while *Felicia* Cass. was nested in a more derived clade containing *Oritrophium*. This indicates that the choice of outgroups will be critical. Previous phylogenetic analyses based on molecular data (Jansen and Kim 1994, Kim and Jansen 1995, Wagstaff and Breitwieser 2002, Funk et al. 2005) resolved Anthemideae, Calenduleae and Inuleae as closest to Astereae, and therefore were appropriate for phylogenetic studies of *Chiliotrichum* group. Thus outgroup was represented by six accessions of ITS and five of ETS sequence data that were obtained from GenBank. The complete list of taxa included herein is given in the table 3.2.

Character Sampling. Both ITS and ETS sequence data, independently and combined, were employed in this research. As discussed previously (chapter 2) both regions of the nuclear ribosomal DNA were shown to be successful in phylogenetic analyses of Asteraceae at generic and species level (Baldwin et al. 1995, Baldwin and Markos 1998, Bena et al. 1998,

Morgan 1997, Linder et al. 2000, Noyes 2000, Urbatsch et al. 2000, 2003, Lowrey et al. 2001, Markos and Baldwin 2001, Cross et al. 2002, Plovanich and Panero 2004, Martins and Hellwig 2005).

The ITS data matrix included 55 accessions representing 31 genera and 50 species; 26 sequences were presented for the first time, and remaining 24 were obtained from the GenBank. The ETS data matrix included 42 accessions from 25 genera and 35 species; 36 accessions were presented for the first time and six were acquired from the GenBank. Taxon sampling between ITS and ETS data matrices was incongruous because of the difficulties in amplifying either the ETS or the ITS in a number of taxa. Consequently, the combined data matrix included a total of 36 accessions representing 23 genera and 32 species. Species, vouchers and accession numbers are given in table 3.2.

DNA Extraction, Amplification (PCR) and Sequencing. Each species was sampled from two different populations, when possible. Extraction of the total genomic DNA, amplification and sequencing of the ITS and ETS regions followed protocols described in chapter 2.

Sequence Alignment and Analysis. Sequences were edited in Sequencher™ 4.1 (Gene Codes, Corporation, Ann Arbor, MI). Initial sequences were aligned using Clustal X ver. 1.81 (Thompson et al. 1997) and manually adjusted in MacClade 4.08 (Maddison and Maddison 2005) as needed. New sequences were added to the existing matrices and aligned visually. The final aligned data matrices were subjected to simple neighbor-joining analyses. Obtained trees were then used to order the taxa according to their phylogenetic similarity, and then realigned manually, in an attempt to minimize homoplasy. The boundaries of the ITS and

Table 3.2. List of taxa, collection information and GenBank numbers. References for sequences obtained from other authors are given.

SPECIES LIST	LOCALITY	COLLECTOR (HERBARIUM)	GENBANK # ITS	GENBANK # ETS	REFERENCE
Outgroup Taxa					
ANTHEMIDEAE					
Argyranthemum foeniculaceum (Willd.) Webb ex Sch. Bip.		9 (ORT)			Francisco-Ortega et al. 1997
ITS1			AF155270		
ITS2		"	AF155307		"
Argyranthemum foeniculaceum (Willd.) Webb ex Sch. Bip.				AF123546	Linder et al. 2000.
Argyranthemum winteri (Svent.) Humphries				AF123545	Linder et al. 2000.
Artemisia bigelovii Gray	USA: Utah: Emery Co.	A. Kornkven 300 (OKL)			Kornkven et al. 1998
ITS1			AF060469		
ITS2	"	"	AF061385		"
**Matricaria recucita* L.		MATI2 (J. L. Hudson Seedsman, Redwood City, CA, USA)			Francisco-Ortega et al. 1997
ITS1			AF155271		
ITS2		"	AF155308		"
**Matricaria matricarioides* (Less.) Porter		Bayer AB-95005 (CANB)		AF319724	Bayer et al. 2002
CALENDULEAE					
Calendula officinalis L.	cult. New Zealand, Christchurch	Breitwieser 2129 (CHR)	AF422114		Wagstaff et al. 2002
Calendula officinalis L.	South Africa: Commercial source	Bayer GH-95009 (CANB)		AF319684	Bayer et al. 2002.
Osteospermum fruticosum (L.) Norl.	cult. New Zealand, Christchurch	Breitwieser 2131 (CHR)	AF422131		Wagstaff & Breitwieser 2002

table 3.2 continued

INULEAE					
Inula helenium L.	cult. Bergius Botanical Garden, Stockholm		U84777		Eldenas et al. 1998
Inula helenium L.	Eurasia: Commercial source	Bayer GH-95013 (CANB)		AF319716	Bayer et al. 2002
Ingroup Taxa					
ASTEREAE					
Subtribe Asterinae					
Galatella linosyris Reichb.	Austria	Seiller s.n (F)	DQ478987	DQ479043	
Subtribe Bellidinae					
Bellidiastrum michelii Cass.	France: Valais: Bourg. St. – Pierre	S. Castroviejo 11118 (MA)	AF494011		Fiz et al. 2002
Subtribe Felicinae					
Amellus microglossus DC.	South Africa: Oranjemund: N. W. Cape; 700 m alt.	M. F. Thompson 159 (MO)	DQ478995	DQ479052	
Amellus strigosus (Thunb.) Less.	South Africa: E Cape: Port Elizabeth; 20 m alt.	K. A. Dahlstrand 3153 (MO)	DQ478996	DQ479053	
Felicia aethiopica (Burm. f.) Bol. & W. Dod	South Africa: Cape Province	E. Esterhysen 36321(MO)	DQ478997	DQ479054	
Felicia echinata Nees	South Africa: Eastern Cape Province	(LC)	AY193797		Eastwood et al. 2004
Felicia filifolia (DC.) Burtt-Davy ssp. *schaeferi* (Dinter) Grau	South Africa: Northern Cape Province	Bayer and Puttock SAF-96166 (CANB)		AF319703	Bayer et al. 2002
Felicia fruticosa Nichols. ssp. b*revipedunculata* (Hutchinson) Grau	South Africa: Cape Province: Victoria West District; 1300 m alt.	I. H. Hartley 873	AY193795		Eastwood et al. 2004
Zyrphelis decumbens (Schltr.) Nesom	South Africa: Worcester Div.: Old Toll Gate	F. M. Leighton 1978 (NY)	DQ478998	DQ479055	
Subtribe Grangeinae					
Grangea maderaspatana (L.) Poir	Sri Lanka: North western Province Kurunegala District; 300 m alt.	D. D. Socjarto 4840 (F)		DQ479056	

table 3.2 continued

Grangea maderaspatana (L.) Poir.	Thailand: Chiang Mai	J.F. Maxwell 90-218 (MO)	F046951		Noyes & Rieseberg 1999
Subtribe Hinterhuberinae					
***Celmisia* group**					
Celmisia asteliifolia Hook. f.	Australia: Mt. Field	8904280 (CBG)	AF497702		Cross et al. 2002
Celmisia bellidioides Hook. f.	N. Zealand: South Island: Mount Cook National Park; 43 44 S 170 07 E.	A. Strid 22333 (MO)	DQ479099	DQ479121	
Celmisia lateralis Buchan.	New Zealand: South Island: Paparoa; 800-900 m alt.	A. Strid 22224 (MO)	DQ479100	DQ479122	
Celmisia mackaui Raoul	New Zealand: Akaroa	514149 (CHR)	AF422115		Cross et al. 2002
Celmisia tomentella M. Gray & Given	Australia: Swindlers Ck.	9501182 (CBG)	AF497705		Cross et al. 2002
Damnamenia vernicosa (Hook. f.) D. G. Given	New Zealand: Campbell Island	30823 (CHR)	AF422119		Cross et al. 2002
Pachystegia insignis Cheesem. [1]	New Zealand: Waihopai River (cult at Lincol)	514143 (CHR)	AF422132		Cross et al. 2002
Pachystegia isignis Cheeseman [2]	N. Zealand: Molesworth Station.	J. Urlich s.n. (MO)	DQ479101	DQ479123	
Pleurophyllum criniferum Hook.f.	New Zealand: Campbell Island	542582 (CHR)	AF497700		Cross et al. 2002
Pleurophyllum hookeri Buchanan	Australia: Tasmania: Macquarie Island	J. R. Croft 10401(MO)	DQ479102	DQ479124	
Pleurophyllum speciosum Hook.f.	New Zealand: Campbell Island	536195 (CHR)	AF422133		Cross et al. 2002
***Chiliotrichum* group**					
Chiliophyllum andinum Cabr.	Argentina: De San Juan: Angaco: Sierra de Pie de Palo; 1950-2050 m alt.	A. T. Hanziber et al. 23690 (F)	DQ479103	DQ479125	
Chiliophyllum fuegianum O. Hoffman	Argentina: Neuquen: Los Lagos; 1000 m alt.	E. Gonzales 775 (MO)	DQ479104	DQ479126	
Chiliotrichiopsis keideli Cabrera		P. Simon & M. Bonifancino 520	DQ479105	DQ479127	

table 3.2 continued

Chiliotrichum diffusum (Forst.) Reiche	Argentina: Santa Cruz: Parque Nacional Los Glaciares	M. Bonifancino 520 (USA)	DQ479106	DQ479128	
Chiliotrichum rosmarinifolium Less. [1]	Argentina: Neuquén	M Bonifancino 336 (USA)	DQ479107	DQ479129	
Chiliotrichum rosmarinifolium Less. [2]	Chile: Coihaique: Reserva Forestal Cerro Castillo.	Troels Myndel Pederson 14383 (MO)		DQ479130	
Lepidophyllum cupressiforme (Lam.) Cas. [1]	Argentina: Santa Cruz: Cañadon Boliche	M. Bonifancino 600 (USA)	DQ479108	DQ479131	
Lepidophyllum cupressiforme (Lam.) Cas. [2]		M. Bonifancino 785	DQ479109	DQ479132	
Llerasia lindenii Triana	Peru: Farrenafe: Lambayegua: Laguna Tembladera; 3100 m alt	A. Sagástegui et al. 12776 (F)	DQ479110	DQ479133	
Llerasia macrocephala (Rusby) Pruski	Bolivia: La Paz: Nor Yungas; 3000 m alt.	J. C. Solomon 10716 (F)	DQ479111	DQ479134	
Llerasia sp.	Ecuador: Cotopoxi - Tangurahua: Paramo de Jaramillo; 4100 m alt.	J. Jaramillo 7383 (F)	DQ479112	DQ479135	
Nardophyllum bryoides (Lam.) Cabr. [1]		M. Bonifancino 763	DQ479113	DQ479136	
Nardophyllum bryoides (Lam.) Cabr. [2]	Argentina: Santa Cruz: Lago San Martin: Brazo de la Lancha	M. Bonifancino 515 (USA)	DQ479114	DQ479137	
Nardophyllum bryoides (Lam.) Cabr. [3]			AF511592		Andrus et al. 2002
Nardophyllum chiliotrichoides (J. Rémy in Gay) A. Gray		M. Bonifancino 679	DQ479115	DQ479138	
Oritrophium hieracioides (Wedd.) Cuatr. [1]	Bolivia: La Paz.	J.C. Solomon 16570 (MO)	AF046946		Noyes & Rieseberg 1999
Oritrophium hieracioides (Wedd.) Cuatr. [2]	Bolivia: La Paz: Murillo: Zongo Valley; 4000 m alt.	J. C. Solomon, 13145 (F)	DQ479116	DQ479139	
Oritrophium peruvianum (Lam.) Cuatr. [1]	Ecuador: Loja: road Jimbura-Zumba, 3480-3600 m alt.	P. M. Jorgensen 2266 (MO)	x	DQ479140	
Oritrophium peruvianum (Lam.) Cuatr. [2]	Ecuador: Pichincha; 3960 m alt.	J. Jaramillo et al. 21020 (QCA)	DQ479117	DQ479141	

table 3.2 continued

Pteronia camphorata L. var. *camphorata*	S. Africa: Cape Province: Namaqualand, Khamiesberg; 1550 m alt.	K. Bremer 496 (MO)	DQ479118	DQ479142	
Pteronia incana (Burm.) DC.	South Africa: Cape.	H. Joffe 850 (MO)	AF046947		Noyes & Rieseberg 1999
***Madagaster* group**					
Madagaster madagascariensis (Humbert) Nesom	Madagascar: Central Madagascar: Mt. Ibity; 1700 m alt.	B. DuPuy & D. DuPuy MB302 (MO)	DQ479031	DQ479087	
Madagaster mandrarensis (Humbert) Nesom	Madagascar: Antsiranana Reserve Naturelle Marsojejy; 1100-1300 m alt.	J. S. Miller 3525 (MO)	DQ479032	DQ479088	
***Novenia* group**					
Novenia acaulis (Wedd. ex Benth) Freire & Hellwig [1]	Peru: San Miguel: Cajamarca: Las Lagunas; 4000 m alt.	A. A. Sagástegui 15719 (NY)	DQ479119	DQ479143	
Novenia acaulis (Wedd. ex Benth) Freire & Hellwig [2]	Peru: Cajamarca: San Miguel: Alvededores de El Tingo (Aqua Blanca); 3100 m.	J. L. Mostacero 1129 (TEX)	DQ479120	DQ479144	
***Olearia* group**					
Olearia argophylla (Labill.) Benth.	Australia: Southeastern Australia		AF247064		Lowrey et al 2001
Olearia pannosa Hook.	Australia: Strathalbyn	24061 (UNSW)	AF247065		Cross et al. 2002
Unknown Subtribe					
Commidendrum rotundifolium (Roxb.) DC.	St. Helena: Pounceys.	(E)	AY193793		Eastwood et al. 2004
Doellingeria umbellata (P. Mill.) Nees	USA: Michigan: Chippewa Co.	Schmidt & Merello 1060 (TEX)	AF477625	AF477754	Urbatsch et al. 2003
Eucephalus elegans Nutt.	USA: Oregon: Baker Co., Wallowa Mountains	V. Karaman 101 (LSU)		x	
Eucephalus glabratus (Greene) Greene	USA: California: Siskiyon Co.: Scott Mountains	V. Karaman 122 (LSU)	DQ479041	DQ479098	

table 3.2 continued

Melanodendron integrifolium (Roxb.) DC.	St. Helena: High Peak; 720 m alt.	V. Thomas 1 (E)	AY193794	Eastwood et al. 2004

* ETS and ITS sequences from different species of the genus that were combined in the ITS/ETS combined data set.

ETS regions were determined using the published sequences available in the GenBank (Noyes and Rieseberg 1999, Markos and Baldwin 2001). Uncorrected pairwise distance of the ingroup taxa and their GC content was calculated using Paup* 4.0b10 (Swoford 2000).

Phylogenetic Reconstruction. Maximum parsimony and Bayesian analyses were performed for ITS and ETS data sets independently, and on the reduced combined ITS/ETS data set. The two datasets were tested for their congruence using the partition homogeneity test (or the incongruence length difference test of Farris et al. 1994) incorporated in Paup* 4.0b10 (Swoford 2000). The partition homogeneity test was implemented using TBR branch-swapping algorithm, simple addition of sequences and saving 200 trees at each replicate.

Maximum parsimony (MP) was performed using Paup* 4.0b10 (Swoford 2002). The heuristic searches used random stepwise taxon addition to obtain starting trees and the tree bisection-reconnection (TBR) branch swapping option, with steepest descent off. Nucleotide characters were unordered and equally weighted, and gaps were treated as missing data. Searches were replicated 10,000 times. Heuristic searches of the ITS dataset were conducted in two steps due to the memory limitations. The initial search was performed saving 100 trees per replicate in order to explore different tree islands. These heuristic searches resulted in a pool of trees that were then used as starting trees in a second heuristic search where the trees were swapped to completion (Maddison 1991). Relative clade support was calculated using bootstrap analyses with TBR branch swapping, 1000 bootstrap replicates each with 10 random addition replicates. Because of the memory constraints, bootstrap support in ITS topology was conducted saving no more than 100 trees per replicate (Mort et al. 2000, Salamin et al. 2003).

Bayesian inference of the three datasets was performed using MrBayes 3.1 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). Likelihood parameters for Bayesian analyses were calculated using MrModeltest 2.2 (Nylander 2004). GTR-I-Γ (for ETS and combined data matrix) and GTR-Γ model (for ITS data matrix) were chosen under AIC criteria (Buckley et al. 2002, Posada and Buckley 2004). The model included six substitution rates, rates followed gamma distribution with four categories (ngamma=4) and sites were assumed invariable (for ETS and combined data matrices) or variable (for ITS data matrix). Specific nucleotide frequencies were not defined *a priori*, instead they were estimated from the data. Two independent runs were done, each starting from an independently generated random tree. Both runs consisted of three incrementally heated and one cold chain. Bayesian analyses were run until the average standard deviation of split frequencies between the two runs dropped below 0.01 (Ronquist et al. 2005). Each Markov chain was run for 0.5-1x10^6 generations, and sampling was done each 200th cycle. To check that the stationarity had been reached, the log-likelihoods of the chains were monitored graphically by plotting them against the generations for each run. 25% of the trees were discarded in burn-in. The resulting 50% majority rule consensus tree was imported and observed in TreeView (Page 1996).

Good bootstrap support (BS) was considered to be ≥70%, moderate support <70% and >50% and poor or no support BS ≤50% (Hills and Bull 1993). For Bayesian clade estimate good support was considered for posterior probabilities (PP) ≥0.90, moderate between PP <0.90 and PP >0.70, low to no support for PP ≤0.70 (Erixon et al. 2003, Randle et al. 2005, Werner et al. 2005).

RESULTS

ITS sequence Data. The length of the ITS region varied from 627 bp in *Galatella lynosiris* to 637 bp in *Felicia echinata* and *Novenia.* Variation in length within Hinterhuberinae was lower, ranging from 632 bp to 637 bp. The ITS-1 region was 249 to 254 bp long, the length of 5.8S subunit was constant at 166 bp and the length of ITS-2 varied from 209 to 217 bp. The aligned matrix of the entire ITS region was 670 bp of which 294 (43.9%) characters were constant, 92 (13.7%) variable characters were parsimony uninformative, and 291 (43.4%) characters were parsimony informative. Alignment required insertion of indels of 1-5 bp long. Because of the missing sequence data for 5.8S unit in the outgroup taxa, that region was excluded in the analyses. The uncorrected pairwise distance among ingroup taxa ranged from 0% to 16%. The GC content varied from 48.8% to 58.2% and its mean overall content was 53.72%.

MP analyses of the ITS data resulted in 1056 most parsimonious trees each 1219 steps long (CI=0.514, RI=0.678, RC=0.348). Bayesian analyses were run for $1x10^6$ generations, sampling each 200^{th} cycle. The resulting lnL values of the remaining trees ranged from -6431.43 to -6451.49. The majority rule consensus tree is given in the figure 3.1.

ITS Trees. The tree topologies recovered in MP and Bayesian analyses were congruent. Five clades were recognized: Felicinae, *Madagaster*-Asterinae-North American, *Chiliotrichum*, *Celmisia* and *Novenia-Oritrophium* (fig. 3.1). The Felicinae clade was the most basal, and it included representatives from the subtribe Felicinae and the two unplaced genera, *Commidendrum* and *Melanodendron*. The *Madagaster*-Asterinae-North American clade includes *Madagaster* from Hinterhuberinae, *Bellidiastrum* and *Galatella* from Bellidinae and Asterinae respectively, and the two unplaced genera that lie in the North

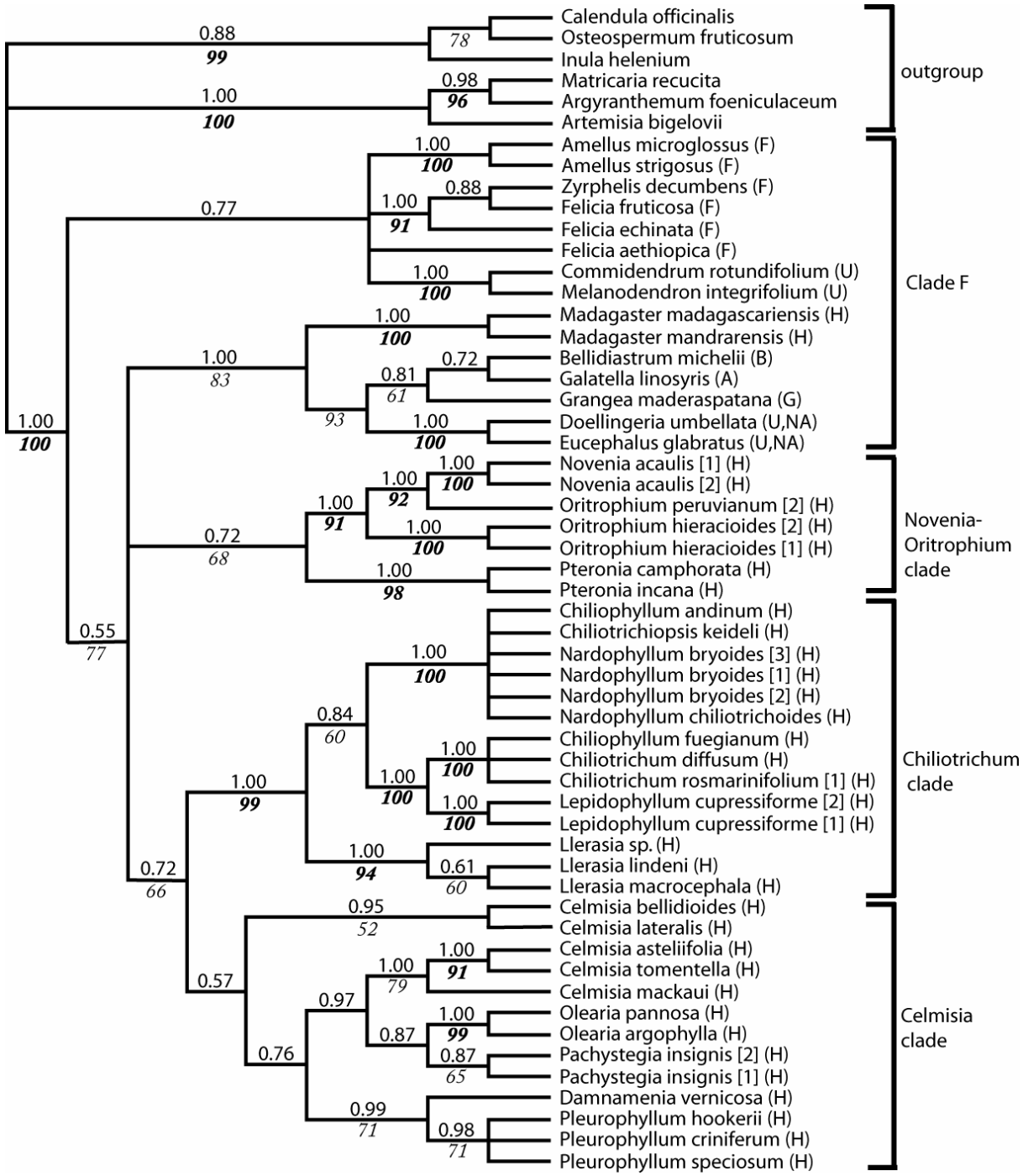


Figure 3.1. Phylogram inferred from Bayesian analyses of ITS data and strict consensus of 1056 most parsimonious trees. Decimal numbers above branches are posterior probability values. Bootstrap support values (≤50% not presented) are given below branches; values ≥90% are in bold. Subtribe names are abbreviated as follow: A=Asterinae; B=Bellidinae; F=Felicinae; G=Grangeinae; H=Hinterhuberinae; NA= North American clade; U=Unplaced.

American clade *sensu* Noyes and Rieseberg (1999). These two clades are arbitrarily designated as clade F for the reasons explained later (see below). The *Chiliotrichum* group (*sensu* Nesom 1994, excluding *Diplostephium*) was not monophyletic. *Oritrophium* and *Pteronia* that are considered within *Chiliotrichum* group were recovered as derived and distantly related to the group. They formed *Oritrophium-Novenia* clade (fig. 3.1) in which *Oritrophium* and *Novenia* grouped in a well supported clade (BS=92%, PP=1.00), that was sister to *Pteronia* (BS=68%, PP=0.72).

The genus *Chiliophyllum* was not monophyletic. *Chiliophyllum andinum* was unresolved in a clade with *Chiliotrichiopsis* and *Nardophyllum* (BS=100%, PP=1.00), and *Chiliophyllum fuegianum* was placed in a highly supported clade with *Chiliotrichum* (BS=100%, PP=1.0) that was sister to *Lepidophyllum* (BS=100%, PP=1.00). *Llerasia* was basal to the remaining *Chiliotrichum* clade. The fifth clade was formed by representatives of genera all distributed in Australia and New Zealand. *Olearia* is a large, polyphyletic genus (Cross et al. 2002). Because the goal of this paper was not to investigate the phylogeny of *Olearia*, the genus was represented with only two accessions obtained from GenBank. Based on the results presented in Cross et al. (2002), we chose two species that were in their "clade II" and were related to *Chiliotrichum*. Resulting topologies resolved *Celmisia* as sister to *Olearia* and *Pachystegia* Cheeseman (PP=1.00) and all of them were sister to the *Damnamenia-Pleurophyllum* clade (*Celmisia* group, fig. 3.1).

ETS Sequence Data. Because of the lack of conserved primer regions, various primer pairs were used for amplifying and sequencing the 3' ETS region. Resulting sequences were different in length; hence, to reduce the amount of missing data, 120 characters were removed from the 5' end of the original matrix. The length of these truncated sequences varied from

441 bp in *Zyrphelis decumbens* to 454 bp in *Novenia acaulis*. The 3' end of the outgroup sequences could not be aligned unambiguously with the ingroup taxa. Therefore, 82 characters at the 3' end of the region were removed before submitting for phylogenetic analyses. Thus the final aligned matrix of the 3' ETS region was 388 bp long; 131 (33.8%) characters were constant, 42 (10.8%) were autopomorphies, and 215 (55.4%) were parsimony informative. The length of the indels varied from 1-9 bp. The uncorrected pairwise distance between ingroup taxa ranged from 0% to 21.4%. The overall GC content varied from 43.4% to 51.6% and the mean content was 48.54%.

MP analyses of the ETS data resulted in 66 most parsimonious trees each 714 steps long, on one tree island (CI=0.585, RI=0.751, RC=0.440). Bayesian analyses were run for $1x10^6$ generations sampling each 200^{th} cycle. Resulting lnL values of the remaining trees ranged from -3800.94 to -3818.24. The majority rule consensus tree and the strict consensus are given in the figure 3.2.

ETS Trees. Tree topologies recovered in MP analyses showed lower resolution and clade support, but overall they were highly similar to the topologies inferred from the ITS data sets. MrBayes inferred topology resolved three sister clades (PP=1.00). The *Celmisia* clade was basal. The *Chiliotrichum*, *Novenia-Oritrophium* clades and the clade containing Felicinae, *Madagaster*, Asterinae, Bellidinae, Grangeinae and North American accessions (clade F) were well supported (PP>0.99, BS). *Pteronia* was unresolved within this clade. Felicinae were not basal as in the topologies based on ITS data, but they were nested within the F clade.

Combined Data. Because the ITS and ETS datasets could not be matched for the species, the combined ITS/ETS data matrix included reduced number of taxa. The aligned

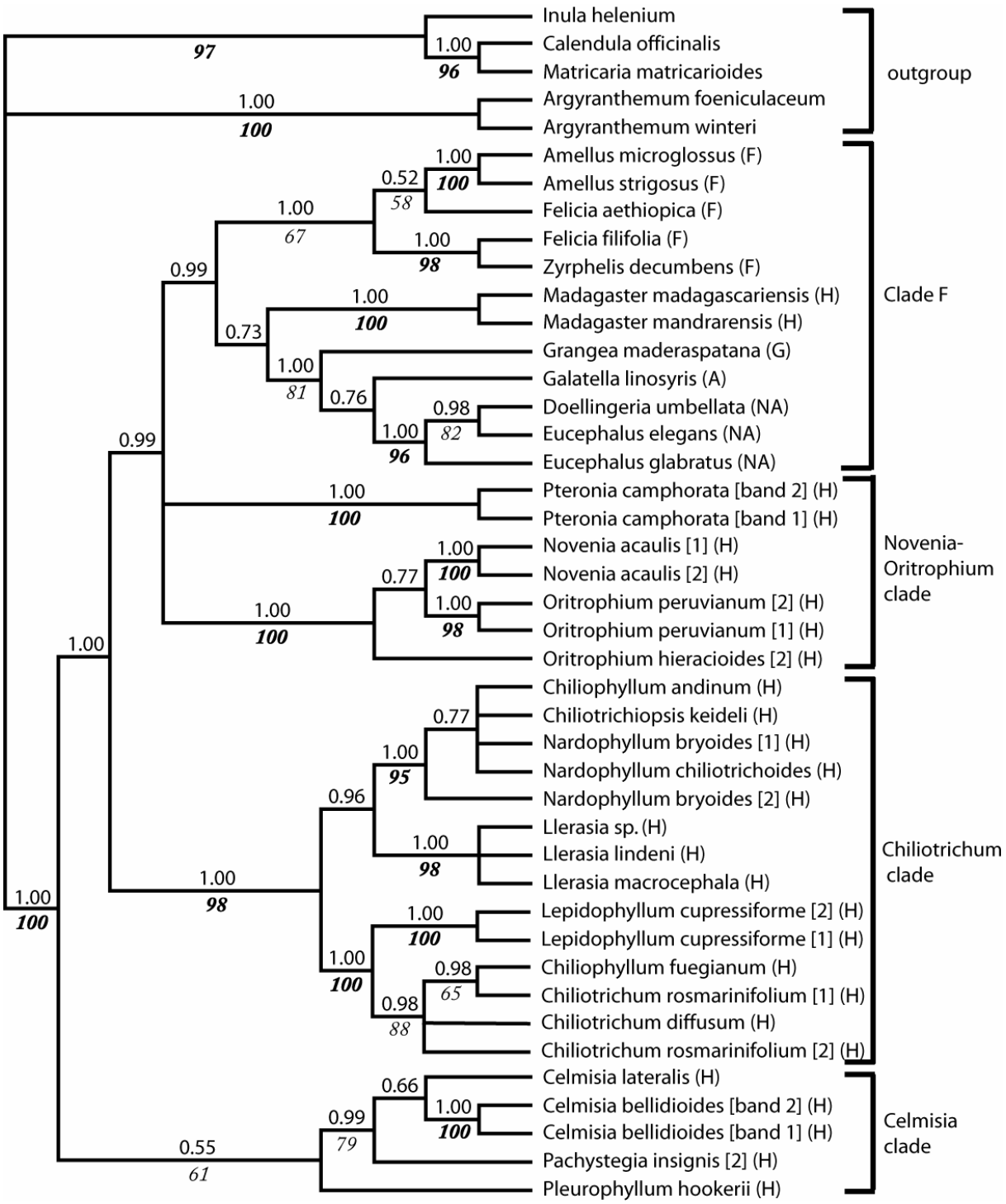


Figure 3.2. Phylogram inferred from Bayesian analyses based on ETS data and strict consensus of 66 most parsimonious trees. Decimal numbers above branches are posterior probabilities. Bootstrap support values (≤50% not presented) are given below brunches; values ≥90% are in bold. Subtribe abbreviations are as in figure 3.1.

matrix was 887 bp long of which 293 (33%) characters were constant, 161 (18.2%) variable characters were parsimony-uninformative and 433 (48.8%) characters were parsimony informative. The partition homogeneity test (Farris et al. 1994) rejected the congruence of the two datasets (p=0.001). When the outgroup taxa were excluded from the analyses, the congruence of the two data sets was supported (p=0.9). Because visual examination of the topologies inferred from the two datasets independently determined that they were congruent, the analyses of the combined data set were conducted using the same outgroup taxa.

Heuristic search using the parsimony optimality criteria resulted in 20 most parsimonious trees each 1624 steps long, from one tree island (CI=0.576, RI=0.674, RC=0.389). The resulting strict consensus of the most parsimonious trees is given in the figure 3.3. Bayesian analyses were run for $0.5x10^6$ generations sampling each 200^{th}-generation. The lnL of the resulting trees varied from -9115.19 to -9132.58. The majority rule consensus of these trees is shown in figure 3.4.

Combined Tree. Resulting topologies inferred from MP (fig. 3.3) and Bayesian (fig. 3.4) analyses differed in the designation of the basal group. The MP analyses resolved Felicinae as the most basal, and MrBayes analyses placed *Celmisia* group as sister to the rest of the ingroup taxa. Another difference was in the placement of *Llerasia* being either basal in the *Chiliotrichum* clade (fig. 3.3) or sister to the *Nardophyllum* lineage (fig. 3.4). The topology recovered in MP analyses was overall congruent with the phylogeny based on the ITS, and the Bayesian phylogeny was congruent with the ETS based topology. Overall clade support was similar to the one recovered on the ITS (fig. 3.1) and ETS (fig. 3.2) topologies. However, in the case of the *Novenia-Oritrophium* clade the support increased from PP=0.77

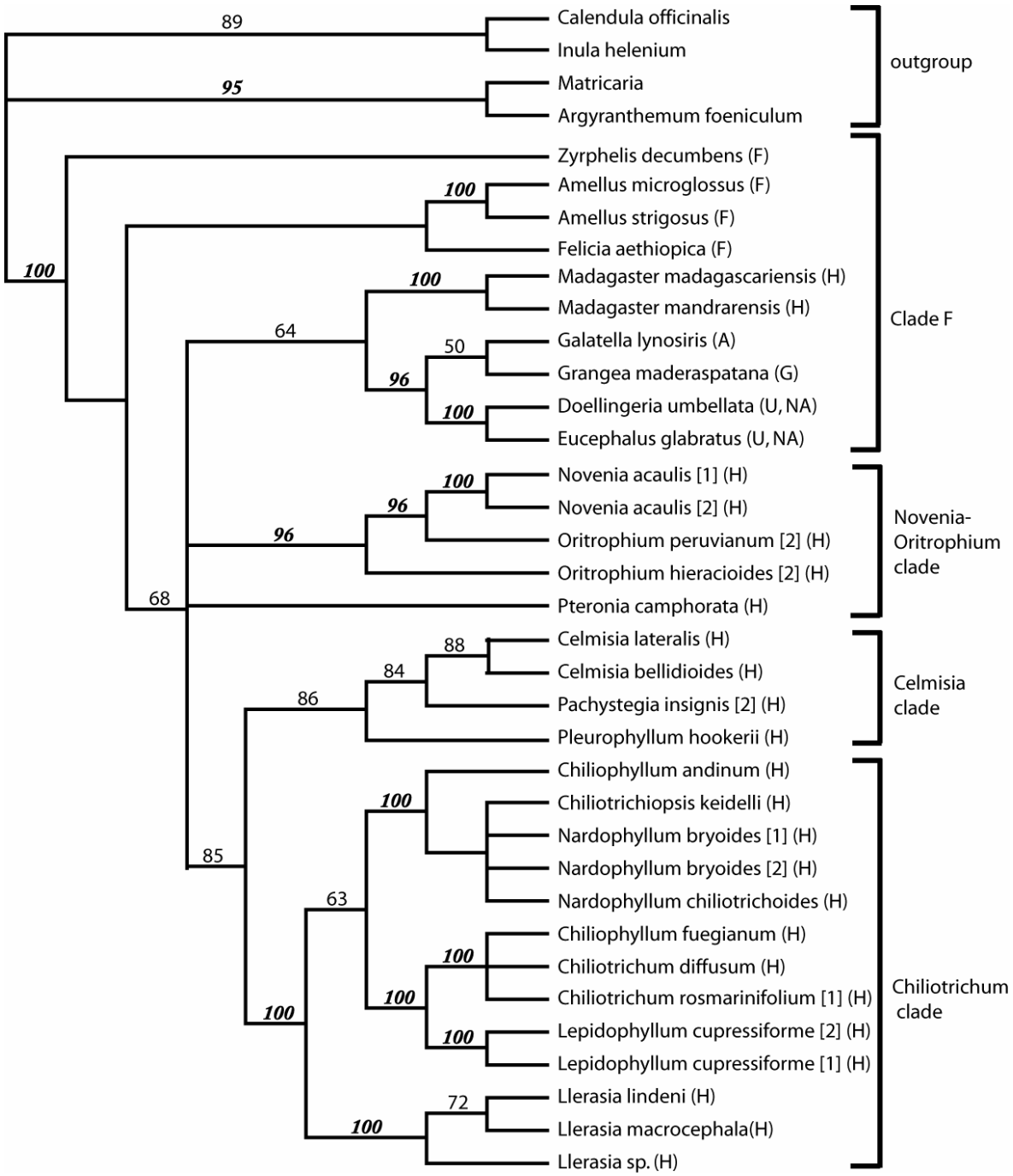


Figure 3.3. Strict concensus tree of 20 most parsimonious trees based on the combined sequence data. Bootstrap values (≤50% not presented) are given above branches; values ≥90% are in bold. Subtribe abbreviations are as in the figure 3.1.

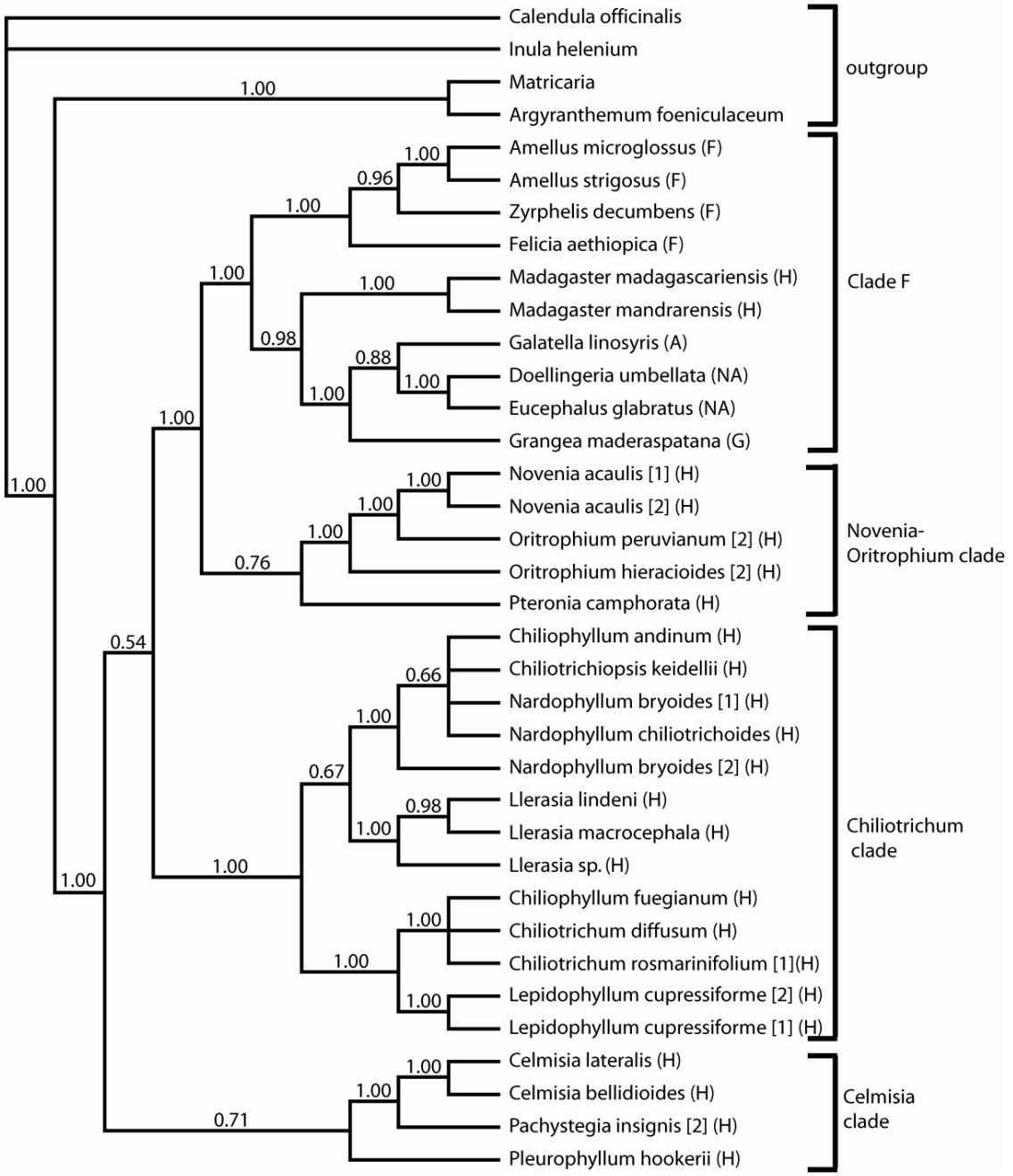


Figure 3.4. Phylogram inferred from Bayesian analyses of combined data. Decimal numbers above branches are posterior probability values. Subtribe abbreviations are as in the figure 3.1.

(fig. 3.2) to PP=1.00 (fig. 3.4) when combining the data, but the support for the basal position of *Llerasia* decreased from PP=0.96 (fig. 3.2) to PP=0.67 (fig. 3.4)

DISCUSSION

Results of the previous phylogenetic analyses based on nuclear ribosomal DNA ITS data (Noyes and Rieseberg 1999, Cross et al. 2002) did not support the monophyly of the *Chiliotrichum* group *sensu* Nesom (1994). One genus, *Diplostephium* was placed in the Southern Hemisphere grade, and thus only distantly related to this group. The phylogenies based on the ITS and ETS sequence data (see chapter 2) placed *Diplostephium* related to the *Hinterhubera* lineage. In addition, close relationship between *Chiliotrichum* and *Oritrophium* was not supported. *Chiliotrichum* was placed as an early diverging lineage within Astereae (Cross et al. 2002) and sister to *Olearia* in part and the *Celmisia* group. *Oritrophium* was basal in the clade containing Felicinae, Podocominae, Lagenophorinae, Baccharidinae, North American Astereae and Hinterhuberinae (Cross et al. 2002). Similar results were confirmed herein. *Oritrophium* grouped with *Novenia* in a clade that was apart from the *Chiliotrichum* clade (Fig 3.1, 3.2, 3.3). The *Chiliotrichum* clade was either sister to the *Celmisia* clade (figs. 3.1 and 3.3) or was nested within clade F (fig. 3.2).

The basal position of the Felicinae that was suggested in the phylogeny of Astereae (Noyes and Rieseberg 1999) was supported only in the Bayesian inferred ITS topology (fig. 3.1). Bayesian analyses of the ETS and combined data sets (figs. 3.2 and 3.4), on the other hand, recovered a more derived position of Felicinae within the clade F, in accord with the results by Cross et al. (2002). Madagascan species of Hinterhuberinae, represented by the genus *Madagaster* had a derived position unrelated to the *Chiliotrichum* group . Instead they

were sister to the clade comprised of Grangeinae, Asterinae and North American Astereae (for more details regarding the phylogeny of this genus see chapter 4).

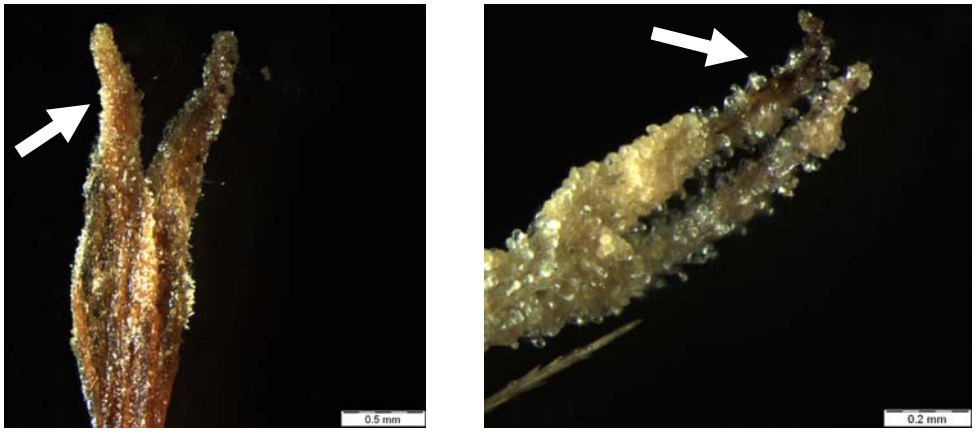
***Oritrophium-Novenia* Clade.** The monotypic *Novenia* was initially considered within the subtribe Gnaphaliinae, tribe Inuleae (Freire 1986, Andenberg 1989), but as noted by Freire (1986) its chromosome number of 2n=18 was atypical for Gnaphaliinae with reported numbers of n=7, 10, 14, 21, 28. Anderberg (1991) and Bremer (1994) included *Novenia* in a group of genera with an unknown position in the subfamily Asteroideae and later, the genus was transferred to Hinterhuberinae based on its rigid leaves, functionally staminate disk florets, reduced pistillate corollas, and multinerved cypselae (Nesom 1994). Up to now placement of this genus within Hinterhuberinae has not been investigated by means of molecular data, and its relationship within the subtribe has remained unresolved. Present molecular analyses resolved *Novenia* in a strongly supported clade related to the herbaceous genus *Oritrophium* (BS>93%, PP>0.77). The two genera are geographically proximal; *Novenia* is distributed in the Andes from Bolivia to northern Peru and Argentina, and *Oritrophium* is found in the Andes of Venezuela, Colombia, Ecuador, Peru, Bolivia, and an additional two species in Mexico. Both genera exhibit adaptations to the climatic and edaphic conditions of puna (*Novenia*) and páramo (*Oritrophium*) ecosystems (Freire 1986, Torres et al. 1996). *Novenia* are small, 1-3.5 cm in diameter, cushiony, cespitose, herbaceous perennials, with numerous sessile capitula in the center of the rosette, while *Oritrophium* includes herbaceous perennials with capitula on long monocephalous stalks. Several synapomorphies support their sister relationship, such as rosulate habit, auxiliary tufts of dense tomentum at the base of leaves and staminate disk florets. The two genera differ in the morphology of the corollas of pistillate florets. The corollas are ligulate in *Oritrophium* and

tubular with much reduced ligule in *Novenia*. Similar reductions of ligules in pistillate ray corollas between related genera are not unusual, and have been observed in the *Hinterhubera* group (see chapter 2) and in Baccharidinae (Hellwig 1996). In the former the tubular, pistillate florets were hypothesized to have been derived from related radiate genera such as *Diplostephium* (see chapter 2). The derived position of *Novenia* within *Oritrophium* suggests similar evolutionary pattern of pistillate corolla reduction.

Morphological similarity between *Oritrophium* and *Celmisia* was recognized by several authors (Schultz-Bipontinus 1856; Solbrig 1960, Nesom 1992, 1993a, 1994). Phylogenies based on ITS and ETS data presented herein confirmed Cuatrecasas' (1997) treatment of the genus as a separate entity that evolved independently of Australian *Celmisia* and "somewhat parallel to *Aster*, *Erigeron*, *Noticastrum*, *Diplostephium* etc. in the American continent". As he pointed out functionally staminate disk florets with sterile ovaries present in *Oritrophium* versus always fertile, hermaphroditic, disk florets in *Celmisia* are characters that separate the two genera. Additionally, most species of *Celmisia* are larger, more robust plants, but a few species are delicate, small plants similar to *Oritrophium*. Thus, similarity in habit, as confirmed by molecular phylogenies, is a result of parallel adaptation to the alpine habitats. Recently two new species of *Oritrophium*, *O. orizabense* Nesom and *O. durangense* Nesom were described from Mexico (Nesom 1992, 1998). This is a remarkable finding because *Oritrophium* has not been found in Central America. Such a disjunct distribution may be attributed to the transport by birds possibly from northern Venezuela (Cuatrecasas 1997).

Molecular data did not support a close relationship of *Oritrophium* and *Novenia* with the *Chiliotrichum* group. This absence of close relationship, however, is not unexpected because *Chiliotrichum* and related genera differ from *Oritrophium* and *Novenia* in several

morphological characters such as shrubby habit, presence of receptacular paleae, and hermaphroditic central florets. Instead, the ITS and combined nrDNA data resolved *Oritrophium* and *Novenia* in a clade sister to *Pteronia* (PP=0.72-0.76, BS= 68%). *Pteronia* is a genus of shrubs endemic to southern Africa, with yellow, discoid capitula and often opposite leaves. Not much is known about the phylogeny of *Pteronia*. Because of its opposite leaves the genus was treated in the *Engleria* group, subtribe Solidagininae (Zhang and Bremer 1993, Bremer 1994), a hypothesis not supported by molecular data (Noyes and Rieseberg 1999, see also chapter 4). A close relationship between *Pteronia* and *Nardophyllum* was observed by Cabrera (1954), but Nesom (1993a) maintained that *Pteronia* was somewhat isolated in the subtribe. Molecular data did not support Cabrera's hypothesis, but suggested a closer relationship of *Pteronia* to *Oritrophium* and *Novenia* than to the *Chiliotrichum* group. Low clade support (PP<0.76, BS<68%) obtained in molecular analyses, however, cannot unequivocally confirm its affiliation to *Oritrophium* and *Novenia*. There is also lack of evident morphological synapomorphies supporting this relationship. Examination of *Oritrophium* and *Pteronia* specimens revealed that disk florets in both genera have lanceolate style branches with abruptly narrowed appendages (fig. 3.5). Similar shape of style branches was also observed in functionally staminate disk florets of *Hinterhubera* and *Laestadia* from *Hinterhubera* group *sensu* Nesom (1994). Style branches observed in *Chiliotrichum* group are linear to linear-lanceolate, typical of those in fertile disk florets. However, for many taxa of Astereae morphology of style branches is not well documented and further detailed observations are needed that may help elucidate relationships among taxa in the *Novenia-Oritrophium* clade.



A **B**

Figure 3.5. Style morphology in *Pteronia* (A) and *Oritrophium* (B). Notice the abruptly narrowed appendages (arrows) in the upper half of the style branches.

***Chiliotrichum–Celmisia* Clade.** Molecular analyses of the ITS and the ETS/ITS combined data supported sister relationship between South American *Chiliotrichum* and Austral-Asian *Celmisia* groups (figs. 3.1 and 3.3, BS=66%, BS=85%, PP=0.72). Such results were congruent with topologies obtained in previous molecular analyses exploring the phylogeny of *Olearia* (Cross et al. 2002). These two groups include genera that are characterized by discoid or radiate capitula, paleate to partially paleate or epaleate receptacles, and hermaphroditic disk florets, except for *Lepidophyllum* where disk florets are functionally staminate.

The *Chiliotrichum* clade includes shrubs distributed in the puna region of Andes with *Llerasia* extending north into Colombia. Pappus of flattened bristles arranged in one to several series is a common feature for this clade. In *Nardophyllum* pappus bristles vary from flattened to terete and widened apically, whereas pappus bristles in *Llerasia* are scabrous, and not flattened. The presence of receptacular paleae also supports the monophyly of this group. The number of paleae varies from completely absent in *Lepidophyllum* and three species of

Nardophyllum, few paleate in *Chiliotrichum* and *Llerasia* to fully paleate in *Chiliotrichiopsis* and *Chiliophyllum*. Variation in their number was also observed at the population level in *Nardophyllum* (Bonifancino and Sancho 2005). *Lepidophyllum* is the only genus in the group with opposite leaves.

Chiliophyllum, a genus that includes three species, two sampled herein, appeared polyphyletic. *Chiliophyllum fuegianum* was grouped with *Chiliotrichum* and *Lepidophyllum* (BS=100%, PP=1.00), while *Chiliophyllum andinum* was sister to *Nardophyllum* and *Chiliotrichiopsis* in a poorly resolved but highly supported clade (BS>95%, PP=1.00). Such topology is supported by morphological characters. Thus, flattened pappus bristles that are widened apically, and densely glandular but almost glabrous cypselae in *Chiliophyllum fuegianum* are also found in *Chiliotrichum* and *Lepidophyllum.* Similarly terete pappus bristles and densely sericeous cypselae in *Chiliophyllum andinum* are observed in *Nardophyllum*. Cypselae in *Chiliotrichiopsis* are likewise densely sericeous, but its pappus is of short scales. Traditionally *Chiliophyllum* was distinguished from *Chiliotrichum* based on the color of ray florets and from *Chiliotrichiopsis* based on the morphology of pappus bristles. Molecular data suggested that these characters are homoplastic for *Chiliophyllum*.

Llerasia is the genus that has the northernmost distribution within the *Chiliotrichum* group, extending north into Colombia. Molecular analyses grouped it with *Chiliotrichum* clade, supporting its segregation from South American *Haplopappus* (Cuatrecasas 1970). Relationships of *Llerasia* within the clade were unresolved. *Llerasia* was either basal in the Chiliotrichum clade (figs. 3.3 and 3.1, BS>99%, PP=1.00), or was sister to *Nardophyllum* (figs. 3.2 and 3.4, PP=0.96 and PP=0.67). Such contradicting placement of *Llerasia* in the ITS and ETS based topologies was probably a reason for a low clade support in the topology

inferred from combined data. *Llerasia* are characterized by discoid capitula and partially paleate to naked receptacle. These two features support sister relationship with *Nardophyllum*. However, *Llerasia* includes small trees or shrubs, often scandent, while *Nardophyllum* are much branched small shrubs. Additionally, cypselae in *Llerasia* are glandular and sometimes shortly pubescent versus densely sericeous in *Nardophyllum*.

IMPLICATIONS FOR THE CHROMOSOME EVOLUTION AND THE ORIGIN OF *CHILIOTRICHUM* AND *NOVENIA-ORITROPHIUM* CLADES

Chromosome numbers in Hinterhuberinae (*sensu* Nesom and Robinson 2006), particularly those in South American taxa are poorly investigated. High level of polyploidy are exhibited in the *Celmisia* group (n=54, 108 in *Damnamenia, Pachystegia, Olearia and Celmisia*; Dawson 2000). The number is not known for *Pleurophyllum*, but based on the molecular data it can be expected to be in the same range. Chromosome numbers of n=27 are reported for *Lepidophyllum, Chiliophyllum fuegianum* (Moore 1981) and *Chiliotrichiopsis keidelii* Cabrera (Hunziker et al. 1989), which are regarded as hexaploids. No data exists for *Nardophyllum, Chiliotrichum and Llerasia*. *Novenia* (n=9) and *Oritrophium* (n=9, 18) are diploids and tetraploids (Turner et al. 1967, Dillon and Turner 1982, Freire 1986). No cytological data are known for *Pteronia*.

Basal position of taxa with woody habit (shrubs and trees) in the ITS based phylogeny by Noyes and Rieseberg (1999) suggested that Astereae may have arisen from a woody ancestor. *Chiliotrichum* and *Celmisia* clades include shrubs and small trees (herbaceous perennial species are found in *Celmisia*) and their basal position in the phylogenies (fig. 3. 4, see chapter 4) supported this hypothesis. Higher levels of polyploidy, such as the one found in *Chiliotrichum* and *Celmisia* groups, have been considered derived in the angiosperms (as discussed in Soltis et al. 2005), thus contradicting the results obtained in the Hinterhuberinae

molecular analyses. This paradox of highly polyploid taxa occupying a basal position within the tribe can be explained by two hypotheses that were, otherwise, proposed to clarify high base chromosome number found in primitive angiosperms (Grant 1981). Lower chromosome numbers such as diploidy and tetraploidy in *Oritrophium* and *Novenia,* may have derived via descending aneuploidy; or secondly, high chromosome numbers (hexaploidy and octaploidy in *Celmisia* and *Chiliotrichum*) may have derived through ancient polyplody and have persisted in these lineages. The geographical distribution of *Chiliotrichum* and *Celmisia* clades and phylogenies of the genera from these two groups favor the second hypothesis as the most parsimonious. *Celmisia* clade is restricted to Australia and New Zealand, while *Chiliotrichum* clade includes genera distributed in the Andes from Tierra del Fuego to Venezuela. Sister relationship of these two groups (although moderately supported) suggests that they may have evolved from the same, probably polyploid ancestors that reached into Australia from South America through Antarctica before the complete separation of the continents in Eocene (some 45-49 million years ago). Available information cannot reliably confirm whether the center of origin for the *Chiliotrichum* and the *Celmisia* groups was in South America or regions of the south Pacific.

An interesting pattern of distribution has been observed within *Chiliotrichum* and *Oritrophium-Novenia* clades. *Chiliotrichum*, *Nardophyllum*, *Lepidophyllum*, *Chiliophyllum*, *Chiliotrichiopsis, Aylacophora* and *Paleaepappus* are restricted to the arid puna regions of southern parts of Chile and Argentina (Patagonia and Tierra del Fuego) extending northward to the region close to the Bolivia-Argentina-Chile border (BAC line, fig. 3.6). *Chiliotrichiopsis peruvianum* Nesom, H. Rob. & Granda is the only species from this group

Figure 3.6. Distribution of genera from *Chiliotrichum* and *Novenia* groups in South America. Arrow indicates location for *Chiliotrihiopsis peruvianum*. All localities are based on the literature data and W3TROPICOS database at Missouri Botanical Garden (http://mobot.mobot.org/W3T/Search/vast.html). Map obtained from Perry-Castañeda Library Map Collection, University of Texas, Austin, TX (http://www.lib.utexas.edu/maps/).

found in southern Peru (the placement of this species within *Chiliotrichiopsis* is questionable, Nesom et al. 2001). *Llerasia*, *Novenia* and *Oritrophium*, based on the available data, are found north from the BAC line. *Llerasia* is distributed in the uppermost montane forest of the Andes in Peru, Ecuador, and Colombia (fig. 3.6), while *Oritrophium* and *Novenia* are restricted to the páramos from the BAC line to Venezuela (fig. 3.6).

Such pronounced limitations in the distribution of these species could be the result of barriers present at the BAC line during the Pleistocene glacial times. The extended lake system of Titicaca and extensive glaciations of the Cordillera Real east of La Paz on the east side, and the glacial lake Minchín which included Lago Poopó and the Salares de Coipasa and Uyuni southwest from Lake Titicaca, probably isolated populations of plants to the north and to the south of it (Simpson 1975).

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CHAPTER 4 PHYLOGENY OF HINTERHUBERINAE WITH BIOGEOGRAPHY AND INFRATRIBAL RELATIONSHIP

The subtribe Hinterhuberinae was established by Cuatrecasas (1969) containing only the genus *Hinterhubera*. Its unique disciform capitula with tubular actinomorphic to zygomorphic deeply lobed corollas of pistillate florets were unusual in existing subtribes at that time. Later Cuatrecasas (1986) expanded the subtribe to include *Westoniella*, *Blakiella* and his newly described genus *Flosmutisia*. Cuatrecasas (1986) redefined the Hinterhuberinae as plants with sterile disk florets, densely glandular and hispid cypselae and ovaries bearing twin-type trichomes. *Blakiella* is exceptional in having glabrous cypselae. However, the ovaries of its sterile disk florets are sparsely pubescent. With the establishment of Hinterhuberinae, the number of subtribes increased to seven, Solidagininae, Grangeinae, Bellidinae, Asterinae, Conyzinae, Baccharidinae and Hinterhuberinae. Grau (1977) considered only two subtribes as good natural groups, Grangeinae and Baccharidinae, and instead dividing the genera into subtribes he arranged them geographically. Cladistic analysis of the tribe Astereae based on morphological data (Zhang and Bremer 1993, Bremer 1994) did not support subtribe Hinterhuberinae as a cohesive clade, and similarly did not resolve the majority of other previously recognized subtribes in Astereae. As a consequence of their work, Astereae were divided into three subtribes, Grangeinae, Solidagininae and Asterinae *sensu lato*. Grangeinae had a basal position in their cladogram. Solidagininae (Homochromeae) principally included genera with yellow disk and ray florets, while Asterinae (Heterochromeae) included genera with yellow disk and white, pink or blue ray florets. Thus, their classification maintained Bentham's (1873) and Hoffman's (1890) homochromous/heterochromous classification of the tribe. Hinterhuberinae (*sensu*

Cuatrecasas 1986), together with Bellidinae, Conyzinae and Baccharidinae, were nested within Asterinae (Heterochromeae) (Zhang and Bremer 1993). However, Nesom (1993a) considered Hinterhuberinae distinct from Asterinae which primarily occur in the Northern Hemisphere. Instead, he redefined and expanded Hinterhuberinae to include mostly shrubby genera, with *Oritrophium* being the only herbaceous member and thus most derived. Without further explanation, Nesom (1993a) excluded *Blakiella, Westoniella* and *Flosmutisia* but subsequently reinstated the latter two and included *Laestadia*, and *Flosmutisia* in the Hinterhuberinae regarding them as the most specialized elements in the subtribe (Nesom 1993b, 1994).

In the treatment of the tribe Astereae based on morphological characters Nesom (1994) identified 29 genera within the subtribe Hinterhuberinae that he classified in seven informal groups (tab. 4.1). There were several published works related to the subtribe Hinterhuberinae since then. Molecular analyses of the ITS sequence data (Noyes and Rieseberg 1999, Roberts and Urbatsch 2003, Urbatsch et al. 2003) suggested exclusion of *Ericameria* from Hinterhuberinae. Two monotypic, Patagonian genera, *Aylacophora* and *Paleaepappus*, were reinstated from within *Nardophyllum* based on receptacle and pappus morphology (Bonifancino and Sancho 2001). *Nardophyllum scoparium* Phil., anomalous within the genus (Nesom 1993d), was the basis for the new genus *Guynesomia* (Bonifancino and Sancho 2004). And finally, *Blakiella* was transferred from Podocominae back to Hinterhuberinae (Nesom and Robinson 2006). Currently the subtribe includes 32 genera distributed in South and Central America, southern Africa, Madagascar, Australia, New Zealand and its subantarctic islands, and two genera occur in Mexico (tab. 4.1). Hinterhuberinae are characterized by a generally shrubby habit, dense, and persistent

Table 4.1. List of genera from Hinterhuberinae according to Nesom and Robinson (2006) and their distribution. Informal classification into groups is based on Nesom (1994). Unplaced genera - *Blakiella* was once excluded from Hinterhuberinae, G*ynesomia* is a newly described genus with relationship outside *Chiliotrichum* group and *Printzia* is transferred from Inuleae to Hinterhuberinae.

GROUP	GENUS [number of species]	DISTRIBUTION
Chiliotrichum	***Aylacophora*** Cabrera [1]	Patagonia
	Chiliophyllum Phil. [3]	Argentina, Chile
	Chiliotrichiopsis Cabrera [4]	Argentina, Chile, Peru
	Chiliotrichum Cass. [2]	Argentina, Chile
	Diplostephium H.B. & K. [ca. 80]	Chile, Peru, Bolivia, Ecuador, Colombia, Panama, Costa Rica
	Lepidophyllum Cass. [1]	Patagonia
	Llerasia Triana [14]	Bolivia, Peru, Ecuador, Colombia
	Nardophyllum Hook & Arn. [5]	Argentina, Chile, Bolivia
	Oritrophium (H.B. & K.) Cuatr. [20]	Peru, Bolivia, Ecuador, Colombia, Venezuela, Mexico
	Paleaepappus Cabrera [1]	Patagonia
	Pteronia L. [ca.80]	South Africa, Zimbabwe
Hinterhubera	***Aztecaster*** Nesom[2]	Mexico
	Floscaldasia Cuatr. [2]	Ecuador, Colombia
	Flosmutisia Cuatr. [1]	Colombia
	Hinterhubera Sch. Bip. [8]	N Chile, Colombia, Venezuela
	Laestadia Kunth [6]	Peru, Bolivia, Ecuador, Colombia, Venezuela, Hispaniola, Costa Rica
	Parastrephia Nutt. [5]	Peru, Chile, Argentina
	Westoniella Cuatr. [6]	Costa Rica, Panama
Novenia	***Novenia*** Freire [1]	Peru, Bolivia, Argentina
Madagaster	***Madagaster*** Nesom [5]	Madagascar
	Mairia Nees [14]	South Africa
	Rochonia DC. [4]	Madagascar
Celmisia	***Achnophora*** F. Muell. [1]	South Australia

table 4.1 continued

	Celmisia Cass. [ca. 60]	New Zealand, South Australia, Tasmania
	Damnamenia Given [1]	Auckland and Campbell Islands
	Olearia Moench in part	New Zealand
	Pachystegia Cheeseman [1]	New Zealand
	Pacifigeron Nesom [1]	French Oceania
	Pleurophyllum Hook. f. [3]	Sub-Antarctic Islands of New Zealand
Olearia	***Olearia*** Moench in part	Australia
Remya	***Remya*** Hillebrand [3]	Hawaii
Unplaced	***Blakiella*** Cuatr. [1]	Colombia, Venezuela
	Guynesomia Bonifancino & Sancho [1]	Chile
	Printzia Cass.	southern Africa

tomentum, evergreen, coriaceous leaves, heterogamous heads, and paleate or epaleate receptacles. There is a tendency towards ligule reduction in the pistillate ray flowers, and in the sterility of the disk floret ovaries. Cypselae are mostly semi-terete, multinerved, with glandular surfaces and pappus is usually of bi-seriate persistent bristles of more or less even length. Dioecy, otherwise typical for Baccharidinae, is present in the genus *Aztecaster* from Mexico.

BIOGEOGRAPHY

Two main questions concerning Hinterhuberinae biogeography are when and where did the subtribe originate. Reliable fossil data for Hinterhuberinae and the family Asteraceae are restricted only to pollen. In the absence of macrofossils answers to these questions must be extracted from the present geographic distribution of the taxa and the geological history of the areas.

Estimates of the time of the origin of Asteraceae vary from Miocene to Cretaceous or earlier (Turner 1977). The molecular clock comparisons suggest that the Asteraceae originated during the mid-Eocene and rapid divergence of major tribal lineages occurred during the Oligocene (Kim et al. 2005). Pollen data indicate that most of the tribes of the family were in existence by the end of Oligocene (22-25 my BP), a period that was followed by rapid evolution of the family and increase in the species number probably in response to the worldwide aridization of the climate (Raven and Axelrod 1974). The place of origin for the family was hypothesized to be in northern South America or northern Andes (Raven and Axelrod 1974, Turner 1977), in the Pacific area and South America, in particular Brazil, excluding the Amazon Basin (Bremer 1992), or in southern South America (Stuessy et al. 1996).

Present distribution of Hinterhuberinae primarily in the Southern Hemisphere (South America, Africa, Madagascar and Australasia) suggests that the ancestors of Hinterhuberinae were in existence at least by the early Tertiary, when the South Atlantic Ocean opened separating South America from Africa. Dispersal between the continents would have been possible via continental routes or across island bridges that existed between the continents until the late Eocene - early Oligocene. Distribution between South America and Africa was possible via three routes: over the Falkland Plateau, along the Walvis-Rio Grande Rise, and the northern proximity with hundreds of islands that acted as stepping-stones between Brazil and West Africa (Tarling 1980). Direct migration of flora and fauna between South America and Australia through Antarctica was possible in Eocene and most likely continued through Oligocene, approximately some 35 my BP (Raven and Axelrod 1972). The final break between South America and Antarctica happened in the Oligocene, about 27 my. BP.

Separation of Madagascar and India from Africa began some 160 my. BP and direct migrations between Africa and Madagascar may have been possible about 100 my BP. Biogeographical data suggest that the origin of Hinterhuberinae may have been in Madagascar, South America or Australasian regions (Nesom 1994).

In South America the Andes began uplifting about 65 million years ago in the upper Cretaceous or lower Tertiary with the final uplift near the end of the Pliocene or into the Pleistocene, some 4-5 million years ago (Simpson 1975). However, páramo and puna environments have been available for plant colonization on an extensive scale only since the Quaternary, some 2-3 million years ago. Páramo and puna ecosystems are in the form of "islands" isolated by low valleys that became more pronounced as the mountains were progressively raised in the Tertiary. Such systems have been, and still are, important dispersal barriers for high altitude elements. The majority of colonization perhaps occurred during glacial periods when plants were able to disperse more easily because of the increased size of the páramo and reduced distances between such islands (Simpson 1975).

The youth of the páramo and puna ecosystems is evidenced by the presence of relatively few endemic or near endemic genera, but a high percentage of endemic species (Arroyo et al. 1993, Luteyn 1999). Compared to other high elevation tropical floras of Africa and Malaysia, the páramo flora of South America is the richest overall and has the largest actual number of genera and endemic elements (Smith and Cleef 1988, Luteyn 1999). Three processes have been suggested for the evolution of this diverse flora: 1) "vertical evolution", adaptation of lowland, warm-loving plants (neotropical elements) to high elevation environments, 2) long-distance dispersal of cool-adapted plants from north and south temperate regions, and 3) by speciation through isolation from autochthonous elements.

Evolution of some taxa may have included a combination of processes, e.g. *Diplostephium*, *Espeletia* (Asteraceae) and *Jamesonia* (Polypodiaceae), which are exclusively neotropical, may have developed from an ancient ancestral stock that was growing at lower elevations, while their radiation have been primarily horizontal within high elevation habitats (Simpson 1975). Some others, like *Lagenophora* (Asteraceae) have a subantarctic origin and were probably spread through long-distance dispersal into puna and páramo vegetation of the Andes (Cabrera 1978). Geological history of the Andes and presence of genera that are endemic to the region provide strong support for a hypothesis of recent and rapid speciation and dispersal of the taxa in South American Hinterhuberinae. However, because of the difficulties determining morphologically plesiomorphic character states within the subtribe Hinterhuberinae, the origin of Hinterhuberinae remains uncertain.

RELATION WITH OTHER SUBTRIBES WITHIN TRIBE ASTEREAE

The relationship of Hinterhuberinae to the rest of the tribe is unclear. Presently, they have an unresolved position within the tribe Astereae, together with Baccharidinae, another shrubby subtribe distributed primarily in South America (Nesom 1994). Phylogenetic analyses of the nrDNA data (see chapter 2 and 3) suggested closer relationships of genera from Hinterhuberinae to other subtribes than to each other. Therefore, certain morphological characters of *Hinterhuberinae* shared with other subtribes within the tribe Astereae are a hypothesized result of convergent evolution:

a) ovarian sterility in disc flowers, consistent within the species and sometimes within the genera, occurred independently in a number of subtribes (Grangeinae, Lagenophorinae, Podocominae);

b) dioecy has arisen independently in Baccharidinae and Hinterhuberinae;

c) paleate receptacles are present in genera with scattered affinity, but more or less they are characteristic of generic groups in Hinterhuberinae and Baccharidinae;

d) evolutionary transition from predominantly primitive white ray flowers to yellow has occurred independently in several, essentially white-rayed subtribes, such as Felicinae, Grangeinae, and Hinterhuberinae (Nesom 1993a); the color of the ray corollas can be useful for assessing phylogenetic relationships at the species level, but it is homoplastic at higher taxonomic levels;

e) presence of the dense tomentum on the leaves appears to be symplesiomorphic in the tribe Astereae (Cross et al. 2002);

f) caudate or sagittate anther bases are homoplastic, having evolved in parallel in many tribes outside Astereae (Anthemideae, Plucheae, Gnaphalieae, Calenduleae) and also having been derived independently several times within Astereae (Cross et al. 2002).

The goal of this research was to: 1) assess relationships of the genera within the subtribe Hinterhuberinae; 2) investigate the relationship of the subtribe within the tribe Astereae; 3) critically test the hypothesis of the biogeographic origin of the subtribe; and 4) test the morphological characters for their synapomorphy in the subtribe.

MATERIALS AND METHODS

Taxon Sampling. One hundred and three accessions from 86 genera and 18 subtribes of Astereae were selected for the analyses (tab. 4.2). Because the analyses aimed to investigate generic and higher-order relationships, one accession per genus was deemed sufficient except where genera were resolved polyphyletic in the initial analyses. The subtribe

Table 4.2. List of taxa, collection information and GenBank numbers. References for sequences obtained from other sources are given.

SPECIES LIST	LOCALITY	COLLECTOR (HERBARIUM)	GENBANK ITS #	GENBANK ETS#	REFERENCE
Outgroup taxa					
ANTHEMIDEAE					
**Argyranthemum foeniculaceum* (Willd.) Webb ex Sch. Bip.		9 (ORT)			Francisco-Ortega et al. 1997
ITS1			AF155270		
ITS2		"	AF155307		"
**Argyranthemum foeniculaceum* (Willd.) Webb ex Sch. Bip.				AF123546	Linder et al. 2000.
Argyranthemum winteri (Svent.) Humphries				AF123545	Linder et al. 2000.
Artemisia bigelovii Gray	USA: Utah: Emery Co.	A. Kornkven 300 (OKL)			Kornkven et al. 1998
ITS1			AF060469		
ITS2	"	"	AF061385		"
**Matricaria recucita* L.		MATI2 (J. L. Hudson Seedsman, Redwood City, CA, USA)			Francisco-Ortega et al. 1997
ITS1			AF155271		
ITS2		"	AF155308		"
**Matricaria matricarioides* (Less.) Porter		Bayer AB-95005 (CANB)		AF319724	Bayer et al. 2002
CALENDULEAE					
Calendula officinalis L.	cult. New Zealand, Christchurch	Breitwieser 2129 (CHR)	AF422114		Wagstaff & Breitwieser 2002
Calendula officinalis L.	South Africa: Commercial source	Bayer GH-95009 (CANB)		AF319684	Bayer et al. 2002.

table 4.2 continued

**Osteospermum clandestinum* (Less.) Norl.	Australia: Western Australia	Bayer WA-94070 (CANB)		AF319733	Bayer et al. 2002
**Osteospermum fruticosum* (L.) Norl.	cult. New Zealand, Christchurch	Breitwieser 2131 (CHR)	AF422131		Wagstaff & Breitwieser 2002
INULEAE					
Inula helenium L.	Cult. Bergius Botanical Garden, Stockholm		U84777		Eldenas et al. 1998
Inula helenium L.	Eurasia: Commercial source,	Bayer GH-95013 (CANB)		AF319716	Bayer et al. 2002
Ingroup taxa					
ASTEREAE					
Subtribe Asterinae					
Aster amellus L.	Austria: Wien	A. Heimerl (LSU)		DQ479042	
Aster amellus L.	Russia: N. Caucasus	A. K. Skvortsov s.n.	AF046961		Noyes & Rieseberg 1999
Galatella linosyris Reichb.	Austria:	Seiller s.n (F)	DQ478987	DQ479043	
Kalimeris pinnatifida (Maxim.) Kitam.	Cult. USA: S. Carolina: Columbia	Porter s.n. (USCH)	DQ478988	DQ479044	
Subtribe Astranthiinae					
Geissolepis suaedaefolia B. L. Rob.	Mexico: San Luis Potosi	Guy Nesom 6634 (F)	DQ478972	DQ478957	
Subtribe Baccharidinae					
Archibaccharis androgyna Blake	Mexico: Chiapas: Cerro Mozatal.	D. E. Breedlore 3116 (F)	DQ478989	DQ479045	
Baccharis boliviensis (Wedd) Cabrera	Argentina: San Louis: Coronel Pringles; 1532 m alt.	R. H. Fortunato 5518 (USA)	DQ478992	DQ479048	
Heterothalamus spartioides Hook. & Arn.	Argentina: Rio Negro: Adolfo Alsina.	G. Seijo 1454 (NY)	DQ478993	DQ479049	
Subtribe Bellidinae					
Bellidiastrum michelii Cass.	France: Valais: Bourg. St. – Pierre	S. Castroviejo 11118 (MA)	AF494011		Fiz et al. 2002

table 4.2 continued

Bellis perennis L.	Bolivia: La Paz	J.C. Solomon 8238 (MO)	AF046950		Noyes & Rieseberg 1999
Subtribe Boltoniinae					
Boltonia diffusa Ell.	USA: Texas	V. Karaman-Castro s.n. (LSU)	DQ478973	DQ478958	
Chloracantha spinosa (Benth.) Nesom	USA: Louisiana: Cameron Pa.	D. M. Ferguson 210 (LSU)	AF477635	AF477699	Urbatsch et al. 2003
Subtribe Brachycominae					
Brachycome rigidula (D.C.) G. L. Davis	Australia: Victoria: Mt. Hotham; 1700-1800 m alt.	A. Strid 22025 (MO)	DQ478994	DQ479051	
Subtribe Chaetopappinae					
Chaetopappa effusa (Gray) Shinners	USA: Texas: Kerr Co.	G. Nesom 7510 (LSU)	DQ478974	DQ478959	
Subtribe Chrysopsidinae					
Croptilon divaricatum (Nutt.) Raf.	USA: Texas	Nesom 7470 (UC)	AF251576	AF251634	Markos & Baldvin 2001
Noticastrum marginatum (Kunth) Cuatr.	Peru: Junin: Yauli; 4200 m alt.	D. M. Pearsall 1173 (F)	DQ478975	DQ478960	
Subtribe Conyzinae					
Erigeron bellidiastrum Nutt.	USA: Texas: Roberts Co.	V. Karaman 8 (LSU)	DQ478976	DQ478961	
Erigeron subtrinervis Rydb.	USA: Colorado: Archuleta Co.	V. Karaman 29 (LSU)	DQ478977	DQ478962	
Subtribe Felicinae					
Amellus microglossus DC.	South Africa: Oranjemund: N. W. Cape; 700 m alt.	M. F. Thompson 159 (MO)	DQ478995	DQ479052	
Felicia aethiopica (Burm. f.) Bol. & W. Dod	South Africa: Cape Province.	E. Esterhysen 36321(MO)	DQ478997	DQ479054	
Felicia echinata Nees	South Africa: Eastern Cape Province.	(LC)	AY193797		Eastwood et al. 2004
Felicia filifolia (DC.) Burtt-Davy ssp. *schaeferi* (Dinter) Grau	South Africa: Northern Cape Province	Bayer and Puttock SAF-96166 (CANB)		AF319703	Bayer et al. 2002

table 4.2 continued

Zyrphelis decumbes (Schltr.) Nesom	South Africa: Worcester Div.: Old Toll Gate.	F. M. Leighton 1978 (NY)	DQ478998	DQ479055	
Subtribe Grangeinae					
Egletes liebmannii Sch. Bip..	Mexico: Veracruz: Mun. Tempoal; 50 m alt.	M. Nee & B. F. Hansen 18367 (F)	DQ478978	DQ478963	
Grangea maderaspatana (L.) Poir	Sri Lanka: North western Province Kurunegala District; 300 m alt.	D. D. Socjarto 4840 (F)		DQ479056	
Grangea maderaspatana (L.) Poir.	Thailand: Chiang Mai	J.F. Maxwell 90-218 (MO)	F046951		Noyes & Rieseberg 1999
Nidorella polycephala DC.	South Africa: Town Hill: Natal	J. L. Sidey 3023 (F)	DQ478999	DQ479057	
Plagiocheilus bogotensis (H.B.K.) Weddell	Ecuador: Pichincha; 3180 m alt.	V. Zak 86 (MO)	DQ479001	DQ479059	
Subtribe Hinterhuberinae					
***Celmisia* group**					
Achnophora tatei F. Muell.	Australia: Kangaroo Island	(UNSW)	AF497656		Cross et al. 2002
Celmisia bellidioides Hook. f.	N. Zealand: South Island: Mount Cook National Park	A. Strid 22333 (MO)	DQ479099	DQ479121	
Celmisia lateralis Buchan.	New Zealand: South Island: Paparoa; 800-900 m alt.	A. Strid 22224 (MO)	DQ479100	DQ479122	
Damnamenia vernicosa (Hook. f.) D. G. Given	New Zealand: Campbell Island	30823 (CHR)	AF422119		Cross et al. 2002
Pachystegia isignis Cheeseman	N. Zealand: Molesworth Station.	J. Urlich s.n. (MO)	DQ479101	DQ479123	
Pleurophyllum hookeri Buchanan	Australia: Tasmania: Macquarie Island	J. R. Croft 10401(MO)	DQ479102	DQ479124	
***Chiliotrichum* group**					
Chiliophyllum andinum Cabr.	Argentina: De San Juan: Angaco: Sierra de Pie de Palo; 1950-2050 m alt.	A. T. Hanziber et al. 23690 (F)	DQ479103	DQ479125	

table 4.2 continued

Chiliophyllum fuegianum O. Hoffman	Argentina: Neuquen: Los Lagos; 1000 m alt.	E. Gonzales 775 (MO)	DQ479104	DQ479126
Chiliotrichopsis keideli Cabrera		P. Simon & Bonifancino, 520	DQ479105	DQ479127
Chiliotrichum diffusum (Forst.) Reiche	Argentina: Santa Cruz: Parque Nacional Los Glaciares	M. Bonifancino 520 (USA)	DQ479106	DQ479128
Chiliotrichum rosmarinifolium Less.	Argentina: Neuquén	M. Bonifancino 336 (USA)	DQ479107	DQ479129
Diplostephium ericoides (Lam.) Cabrera	Ecuador: Carchi	R. M. King et all. 10115 (F)	DQ479004	DQ479062
Lepidophyllum cupressiforme (Lam.) Cas.	Argentina: Santa Cruz: Cañadon Boliche	M. Bonifancino 600 (USA)	DQ479108	DQ479131
Llerasia macrocephala (Rusby) Pruski	Bolivia: La Paz: Nor Yungas; 3000 m alt.	J. C. Solomon 10716 (F)	DQ479111	DQ479134
Nardophyllum bryoides (Lam.) Cabr.	Argentina: Santa Cruz: Lago San Martin, Brazo de la Lancha.	M. Bonifancino 515 (USA)	DQ479114	DQ479137
Oritrophium peruvianum (Lam.) Cuatr.	Ecuador: Pichincha; 3960 m alt.	J. Jaramillo et al. 21020 (QCA)	DQ479117	DQ479141
Pteronia camphorata L. var. *camphorata*	S. Africa: Cape Province: Namaqualand, Khamiesberg; 1550 m alt.	K. Bremer 496 (MO)	DQ479118	DQ479142
***Hintehubera* group**				
Aztecaster pyramidatus (B.L.Rob. & Greenm.) G.L.Nesom	Mexico: Oaxaca, Monte Alban; 1850 m.	J. Rzedowski 28828 (TEX)	DQ479008	DQ479064
Floscaldasia hypsophila Cuatr.	Ecuador: Azuay: Cajas National Park; 4400 m alt.	P. Sklenar 2418 (USA)	DQ479009	DQ479065
Hinterhubera imbricata Cuatr. & Aristeg.	Venezuela: Merida: Parque Nac. Sierra Nevada; 4170 m alt.	A. Berg 171A (MERC)	DQ479013	DQ479069
Laestadia muscicola (Sch. Bip.) Wedd.	Venezuela: Barinas: Paramo El Toro; 2480 m alt.	Briceño, B. et al. 2238 (MERC)	DQ479017	DQ479074

table 4.2 continued

Laestadia pinifolia Kunth	Colombia: Boyaca: Paramo de Chita; 3140 m alt.	A. M. Cleef 4754	DQ479018	DQ479075	
Parastrephia quadrangularis (Meyen) Cabrera	Bolivia: La Paz: Pacajes: Caquiaviri; 4200 m alt.	T. Johns 82-30 (F)	DQ479025	DQ479081	
Parastrephia teretiuscula (Kuntze) Cabrera	Bolivia: Potosi: Antonio Quijarro; 3700 m alt.	G. Torrico 507 (USA)	DQ479026	DQ479082	
Westoniella chirripoensis Cuatr.	Costa Rica: Cartago: Cerro de la Muerte.	V. Karaman-Castro 237 (LSU)	DQ479027	DQ479083	
***Madagaster* group**					
Madagaster madagascariensis (Humbert) Nesom	Madagascar: Central Madagascar: Mt. Ibity; 1700 m alt.	B. DuPuy & D. DuPuy MB302 (MO)	DQ479031	DQ479087	
***Novenia* group**					
Novenia acaulis (Wedd. ex Benth) Freire & Hellwig	Peru: San Miguel: Cajamarca: Las Lagunas; 4000 m alt.	A. A. Sagástegui et al. 15719 (NY)	DQ479119	DQ479143	
***Olearia* group**					
Olearia argophylla (Labill.) Benth.	Australia: Southeastern Australia		AF247064		Lowrey et al 2001
Olearia arguta Benth.	Queensland	Cumming 13918 (BRI)	AF247066		Lowrey et al. 2001
Olearia pannosa Hook.	Australia: Strathalbyn	24061 (UNSW)	AF247065		Cross et al. 2002
Olearia ramulosa (Labill.) Benth	Australia: Victoria: Grampians National Park Area; 370 m alt	R. M. King 9722 (F)	DQ479033	DQ479089	
***Remya* group**					
Remya kauaiensis Hillebrand	Hawaii: Kauai	1996249 (BISH)	AF497684		Cross et al. 2002
unplaced					
Blakiella bartsiaefolia (Blake) Cuatr.	Venezuela: Merida: Rangel; 3000 m alt.	H. van der Werff 7707 (USA)	DQ479034	DQ479090	
Guynesomia scoparia (Phil.) Bonif. & G.Sancho	Chile: Elqui: Llanos de Huanta.	M. Bonifancino 820 (LP)	DQ479035	DQ479091	

table 4.2 continued

Subtribe Lagenophorinae					
Keysseria maviensis (H. Mann) Cabrera	USA: Hawai: Maui: Puu Kukui trail. 5000 ft. alt.	S. Perlman 6875 (USA)	DQ479036	DQ479092	
Lagenophora pumila Cheeseman	New Zealand: South island: Boulder Lake Trail.	S. A. Norton 598 (NO)	DQ479037	DQ479094	
Myriactis humilis Merr.	Taiwan: Pingtum Hsien	T. Chiang 141 (MO)	AF046959		Noyes & Rieseberg 1999
Myriactis panamensis S.F. Blake	Panama: Chiriqui	R. J. Schmalzel 1713 (MO)	AF046965		Noyes & Rieseberg 1999
Subtribe Machaerantherinae					
Grindelia nana Nutt.	USA: California: Lassen Co.: Willowa Creek	V. Karaman 128 (LSU)	DQ478979	DQ478964	
Isocoma tenuisecta Greene	USA: Arizona: Cochise Co.: Benson	V. Karaman 145 (LSU)	DQ478980	DQ478965	
Machaeranthera tanacetifolia (Kunth) Nees	USA: New Mexico: Colfax Co.	V. Karaman 18 (LSU)	DQ478981	DQ478966	
Pyrrocoma clementis Rydb.	USA: Utah: Iron Co.: Cedar Breaks	V. Karaman 76 (LSU)	DQ478982	DQ478967	
Rayjacksonia phyllocephala (DC.) Hartman & Lane	USA: California:	D. M. Ferguson (LSU)	DQ478983	DQ478968	
Subtribe Pentachaetinae					
Pentachaeta exilis (Gray) Gray	USA: California: Monterey Co.	Keil 17085 (TEX)	AY171036	AY171004	Roberts & Urbatsch 2003
Subtribe Podocominae					
Kippistia suaedifolia F. Muell.	New South Wales	Pickard 3657 (NSW)	AF497660		Lowrey et al. 2001
Laennecia schiedeana (Less.) G.L.Nesom	Mexico: Oaxaca: Miahuatlan; 2260 m alt.	Hinton s.n. (LSU)	DQ479038	DQ479095	
Minuria integerrima (DC.) Benth.	Queensland	T. Lowrey 1754 (UNSW)	AF247074		Lowrey et al. 2001
Minuria macrorhiza (DC.) Lander	Northern Territory	Adam 23307 (UNSW)	AF247076		Lowrey et al. 2001
Peripleura bicololor (Burbidge) Nesom	Queensland	T. Lowrey 1765 (UNSW)	AF247078		Lowrey et al. 2001

table 4.2 continued

Peripleura diffusa (Burbidge) Nesom	Queensland	T. Lowrey 1760 (NSW)	AF247079		Lowrey et al 2001
Podocoma notobellidiastrum (Griseb.) Nesom	Paraguay: Caazapa	E. Zardini 3009 (MO)	AF046963		Noyes & Rieseberg 1999
Sommerfeltia spinulosa Less.	Uruguay: Salto: Cerros del Boquerón de Ararunguá.	T. M. Pedersen 16281 (NY)	DQ479039	DQ479096	
Tetramolopium pumilum Mattf.	New Guinea	T. Lowrey 1546 (UNM)	AF247092		Lowrey et al. 2001
Tetramolopium vagans Pedley	Queensland	T. Lowrey 1755 (UNSW)	AF247096		Lowrey et al. 2001
Vittadinia sulcata Burbidge	W. Australia	T. Lowrey 1727 (UNSW)	AF247112		Lowrey et al. 2001
Subtribe Solidagininae					
Chrysothamnus molestus (Blake) Anderson	Arizona: Coconino Co.	Anderson 3146 (CAS)	AY170941	AY169738	Roberts & Urbatsch 2004
Eastwoodia elegans Brandegee	USA: California: Kern Co.	Sanders 20427 (CAS)	AY170949	AY169746	Roberts & Urbatsch 2004
Ericameria resinosa Nutt.	USA: Washington: Klickitat Co.	Brooks 20195 (RM)	AY171031	AY170999	Roberts & Urbatsch 2003
Gundlachia riskindii (Turner & Langford) Urbatsch & R.P. Roberts	Mexico: Nuevo Leon.	G. Nesom 7697 (TEX)	AF477686	AF477750	Urbatsch et al. 2003
Petradoria pumila (Nutt.) Greene	USA: Colorado: Mesa Co.	Urbatsch 7889 (LSU)	AY170959	AY169756	Roberts & Urbatsch 2004
Solidago sempervirens L.	Florida: Wakulla Co.	Urbatsch 7590 (LSU)	AF477668	AF477732	Urbatsch et al. 2003
Subtribe Symphyotrichinae					
Canadanthus modestus (Lindl.) Nesom	USA: Oregon: Baker Co.: Wallowa Mountains	V. Karaman 96 (LSU)	DQ478984	DQ478969	
Symphyotrichum vahlii Nesom	Chile: Ultima Esperanza: Torres del Paine		AY193804		Eastwood et al. 2004
Unplaced					
Commidendrum rotundifolium (Roxb.) DC.	St. Helena: Pounceys.	(E)	AY193793		Eastwood et al. 2004
Doellingeria umbellata (P. Mill.) Nees	USA: Michigan: Chippewa Co.	Schmidt & Merello 1060 (TEX)	AF477625	AF477754	Urbatsch et al. 2003

table 4.2 continued

Eucephalus glabratus (Greene) Greene	USA: California: Siskiyon Co.: Scott Mountains	V. Karaman 122 (LSU)	DQ479041	DQ479098	
Eurybia wasatchensis (Jones) Nesom	USA: Utah: Iron Co.: Cedar Canyon	V. Karaman 66 (LSU)	DQ478985	DQ478970	
Ionactis linariifolia (L.) Greene	USA: Louisiana: Rapides Pa.: Kisatchie Natl. Forest	J. Bruser 357 (LSU)	AF477660	AF477724	Urbatsch et al. 2003
Melanodendron integrifolium (Roxb.) DC.	St. Helena: High Peak; 720 m alt.	V. Thomas 1 (E)	AY193794		Eastwood et al. 2004
Oreostemma alpigenum (Torr & Gray) var. *andersonii* (Gray) Nesom	USA: California: Siskiyon Co., Scott Mountains	V. Karaman 123 (LSU)	DQ478986	DQ478971	

*Sequences of ITS and ETS from two different species that were combined in the ITS/ETS data set.

Hinterhuberinae was represented by 28 genera that were sampled previously (see chapters 2 and 3). The ITS data set contained four taxa of *Olearia*, but only one taxon was present in the ETS data set. To assess the relationships of the subtribe within the tribe Astereae, one to eight genera from 17 subtribes were included in the data set. In addition eight genera currently with uncertain position within Astereae (*sensu* Nesom and Robinson 2006) were sampled.

Previous phylogenetic analyses based on molecular data (Jansen and Kim 1994, Kim and Jansen 1995, Wagstaff and Breitwieser 2002, Funk et al. 2005) resolved Anthemideae, Calenduleae and Inuleae as closest to Astereae. Therefore, the outgroup taxa were chosen from these tribes. A total of six accessions of ITS and five of ETS sequence data were obtained for outgroup taxa from GenBank.

Character Sampling. ITS and ETS sequence data, independently and combined, were employed. As discussed previously (see chapter 2) both regions of the nuclear ribosomal DNA were shown to be successful in phylogenetic analyses of Asteraceae at the generic and species level (Baldwin et al. 1995, Morgan 1997, Baldwin and Markos 1998, Bena et al. 1998, Linder et al. 2000, Noyes 2000, Urbatsch et al. 2000, 2003, Lowrey et al. 2001, Markos and Baldwin 2001, Cross et all. 2002, Plovanich and Panero 2004, Martins and Hellwig 2005).

The ITS data matrix included 101 accessions from 87 genera; 14 of these sequences were new. The ETS data matrix included 79 accessions from 71 genera; 16 accessions were new. Beside the attempts to include taxa with available sequences for ITS and ETS regions, the taxon sampling between the two data matrices could not be matched exactly. As a result, the combined data matrix included a total of 77 accessions from 71 genera.

DNA Extraction, Amplification (PCR) and Sequencing. Each species was sampled from two different populations, when possible. Extraction of the total genomic DNA,

amplification and sequencing of the ITS and ETS regions followed protocols described in chapter 2.

Sequence Alignment and Analysis. Reading, editing and alignment of sequences was done as described in chapter 2. Sequences for three outgroup species obtained from GenBank are composite of separate ITS1 and ITS2 sequences, and lack the 5.8S sequence. These taxa are indicated in table 4.2. Because of the lack of ETS sequence data, sequences of *Matricaria* and *Osteospermum* in the combined data set were composed of two different species as indicated in table 4.2. The boundaries of the ITS and ETS regions were determined using the published sequences available in the GenBank (Noyes and Rieseberg 1999, Markos and Baldwin 2001). Uncorrected pairwise distance of the ingroup taxa and their GC content was calculated using Paup version 4.0b10 (Swoford 2000).

Phylogenetic Reconstruction. Maximum parsimony and Bayesian analyses were performed for ITS and ETS data sets independently, and on the combined ITS/ETS data set. The two data sets were tested for their congruence using the partition homogeneity test (or the incongruence length difference test of Farris et al. 1994) incorporated in Paup* 4.0b10 (Swoford 2000). The partition homogeneity test was implemented using TBR branch-swapping algorithm, simple addition of sequences and saving 100 trees at each replicate.

Maximum parsimony (MP) was performed using Paup* 4.0b10 (Swoford 2002). The heuristic searches used random stepwise taxon addition to obtain starting trees and the tree bisection-reconnection (TBR) branch swapping option, with steepest descent off. Nucleotide characters were unordered and equally weighted, and gaps were treated as missing data. Heuristic searches were conducted in two steps due to the memory limitations. The initial searches were replicated 1000 times, saving 20 trees (50 in combined data analyses) per

replicate in order to explore different tree islands. These heuristic searches resulted in a pool of trees that were then used as starting trees in a second heuristic search where the trees were swapped to completion (Maddison 1991). Strict consensus trees were calculated for the trees obtained in each run separately and compared for their incongruence. The second step of the heuristic searches of ETS data was aborted when available memory was exhausted. The strict consensus tree from these analyses was then used as a reverse constraint (Cantino et al. 1998) in a subsequent heuristic searches with 100 random taxon additions and saving no more than 500 trees each replicate. This method allowed search for additional trees with equal or shorter length that were incongruent with the trees already found. To reduce the influence of highly homoplastic sites in the ITS data set, subsequent analyses were performed applying successive approximations weighting (Farris 1969) according to the mean rescaled index (RC). Relative clade support was calculated using bootstrap analyses with TBR branch swapping, 1000 bootstrap replicates with 10 random addition replicates per bootstrap. Because of the memory constraints, bootstrap analysis was conducted saving no more than 100 trees per replicate (Mort et al. 2000, Salamin et al. 2003).

Bayesian inference of the three data sets was performed using MrBayes 3.1 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). Model parameters for Bayesian analyses were calculated using MrModeltest 2.2 (Nylander 2004). GTR-I-Γ (for ITS and combined data matrix) and GTR-Γ model (for ETS data matrix) were chosen under AIC criteria (Buckley et al. 2002, Posada and Buckley 2004). The model included six substitution rates, rates followed gamma distribution with four categories (ngamma=4) and sites were assumed invariable (for ITS and combined data matrices) or variable (for ETS data matrix). Specific nucleotide frequencies were not defined *a priori*, instead they were estimated from

the data. Two independent runs were done, each starting from an independently generated random tree. Both runs consisted of three incrementally heated and one cold chain. Each Markov chain was run for 1-1.6x10^6 generations, and sampling was done each 200^{th} cycle. To check that the stationarity had been reached, the log-likelihoods of the chains were monitored graphically by plotting them against the generations for each run. Twenty five percent of the trees were discarded in burnin. The resulting 50% majority rule consensus tree was imported and observed in TreeView (Page 1996).

Good bootstrap support (BS) was considered to be ≥70%, moderate support <70% and >50% and poor or no support BS ≤50% (Hills and Bull 1993). For Bayesian clade support estimates good support was considered for posterior probabilities (PP) ≥0.90, moderate between PP <0.90 and PP >0.70, low to no support for PP ≤0.70 (Erixon et al. 2003, Randle et al. 2005, Werner et al. 2005).

Constraint Analyses. Constraint analyses testing basal position of Felicinae were conducted on the ETS data set. The tree that forced Felicinae to be basal in the Astereae was created in MacClade v. 4.08. This constraint was then subjected to heuristic searches with parsimony optimality using Paup* 4.0b10 (Swoford 2000). Recovered most parsimonious trees were used to construct the strict consensus tree, that was then compared with non-constrained strict-consensus of the most parsimonious trees.

Biogeography. Biogeographic history was reconstructed using MacClade (Maddison and Maddison 2001). Geographic distribution was treated as an unordered multistate character and plotted onto the tree. Two input trees were chosen randomly from a pool of trees obtained in the Bayesian analyses of each ITS and the combined sequence data. The following ten geographic regions were designated: Eurasia, Africa (excluding southern Africa), southern

Africa, Madagascar, St. Helena, Australia, New Zealand, South and Central America, and North America.

Morphological Character Optimization. Characters were optimized onto one randomly chosen tree obtained from the Bayesian inference of the ITS data. Character state data were determined for all taxa from observations of the herbarium material and from the literature. Characters for each taxon were coded as species rather then as genera because some of the genera appeared polyphyletic. Characters were also scored for the outgroup taxa (Grandcolas et al. 2004). Following characters were evaluated:

1. Habit: (0) herbaceous, (1) woody
2. Receptacular paleae: (0) present, (1) absent
3. Corolla of pistillate floret with ligule: (0) developed, (1) reduced
4. Disk florets: (0) hermaphroditic, (1) functionally staminate

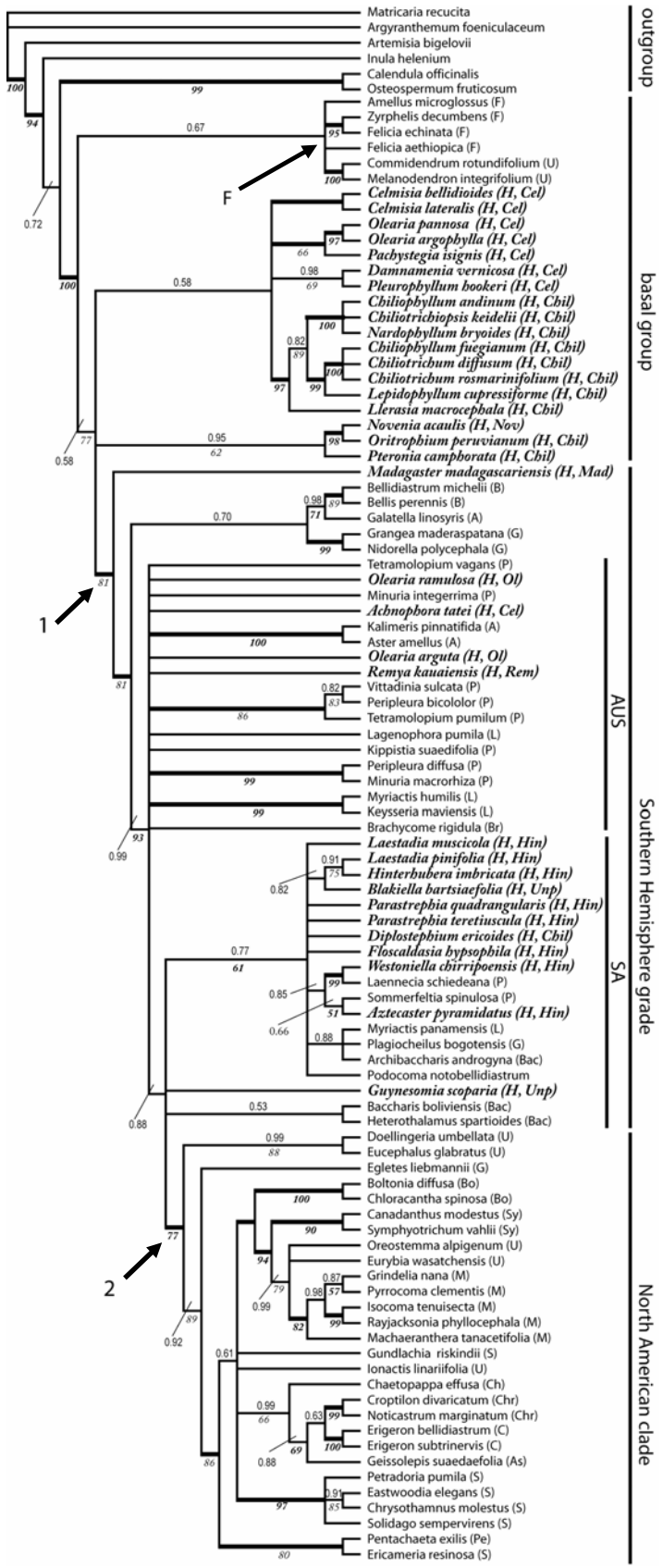
RESULTS

ITS Sequence Data. The length of the ITS region in the ingroup taxa varied from 620 bp in *Symphyotrichum vahlii* to 635 bp in *Zyrphelis decumbens*. The ITS-1 region was 245-255 bp long, the length of 5.8S subunit was constant at 166 bp, and the length of ITS-2 varied from 207 to 216 bp. The aligned matrix of the entire ITS region was 676 bp long of which 119 (17.6%) characters were constant, 77 (11.4%) variable characters were parsimony uninformative while 312 (46.1%) characters were parsimony informative. Alignment required insertion of indels of 1-4 bp long. Because the sequence of the 5.8S region was missing in the outgroup taxa this unit was excluded from the analyses. The uncorrected pairwise distance between ingroup taxa ranged from 0.2% to 19.4%. The GC content varied from 47.5% to 56.3% and its mean overall content was 52.4%.

The initial heuristic search saving only 20 trees per replicate resulted in 1560 trees 2342 steps long. Simple heuristic search using these trees as starting trees which were swapped to completion found 7902 trees of equal length (CI=0.334, RI=0.574, RC=0.192). Branch resolution was very low due to the high level of homoplasy. Three rounds of successive approximations weighting revealed 126 equally parsimonius trees 392.04 steps long (CI=0.565, RI=0.77, RC=0.399), the strict consensus of which was highly congruent with the consensus tree obtained in Bayesian analyses. Bayesian analyses were run for $1.5x10^6$ generations, sampling each 200^{th}-cycle. Resulting lnL values of the retained trees ranged from -12287.51 to -12327.98. Majority rule consensus tree is given on the figure 4.1.

ETS Sequence Data. Because of the lack of the conserved primer regions, various primer pairs were used for amplification and sequencing of the 3' ETS region. Resulting sequences were different in length; hence, to reduce the amount of missing data, 120 characters were removed from the 5' end of the original matrix. The length of these truncated sequences varied from 441 bp in *Zyrphelis decumbens* to 454 in *Novenia acaulis* and *Keysseria maviensis*. The final aligned matrix of the 3' ETS region was 487 bp long; 112 (23%) characters were constant, 100 (20.5%) were autopomorphies and 275 (54.5%) were parsimony informative. The length of the indels varied from 1-4 bp. One indel 80 bp long was autopomorphic for *Gundlachia riskindii*, and therefore removed from the data matrix. The uncorrected pairwise distance between ingroup taxa was somewhat higher compared to the ITS sequences, it ranged from 0.2% to 23.1%. The overall GC content varied from 43.3% to 52.1% and the mean content was 49%.

Figure 4.1. Phylogram inferred from Bayesian analyses and strict consensus of 126 most parsimonious trees based on ITS sequence data. Node 1 - distinguishes basal grade from the others; node 2 - supports the monophyly of the North American clade; AUS-Austral clade; F-Felicinae; SA-South American clade. Numbers above branches are posterior probabilities. Branches with PP=1.00 are thickened. Bootstrap support (<50% not shown) are italic numbers below branches. Values >90% are shown in bold. Subtribe abbreviations in parenthesis are as follows: A-Asterinae, As-Astranthiinae, B-Bellidinae, Bac-Baccharidinae, Bo-Boltoniinae, Br-Brachycominae, C-Conyzinae, Ch-Chaetopappinae, Chr-Chrysopsidinae, F-Felicinae, G-Grangeinae, H-Hinterhuberinae, L-Lagenophorinae, M-Machaerantherinae, P-Podocominae, Pe-Penthachaetinae, S-Solidagininae, Sy-Symphyotrichinae, U-Unplaced. Group affiliation of genera from Hinterhuberinae is as follow: Cel-*Celmisia* group; Chil-*Chiliotrichum* group, Hin-*Hinterhubera* group, Mad-*Madagaster* group, Ol-*Olearia* group, Rem-*Remya* group, Unp-not affiliated.


Matricaria recucita
Argyranthemum foeniculaceum
Artemisia bigelovii
Inula helenium
Calendula officinalis
Osteospermum fruticosum
Amellus microglossus (F)
Zyrphelis decumbens (F)
Felicia echinata (F)
Felicia aethiopica (F)
Commidendrum rotundifolium (U)
Melanodendron integrifolium (U)
Celmisia bellidioides (H, Cel)
Celmisia lateralis (H, Cel)
Olearia pannosa (H, Cel)
Olearia argophylla (H, Cel)
Pachystegia isignis (H, Cel)
Damnamenia vernicosa (H, Cel)
Pleurophyllum hookeri (H, Cel)
Chiliophyllum andinum (H, Chil)
Chiliotrichiopsis keidelii (H, Chil)
Nardophyllum bryoides (H, Chil)
Chiliophyllum fuegianum (H, Chil)
Chiliotrichum diffusum (H, Chil)
Chiliotrichum rosmarinifolium (H, Chil)
Lepidophyllum cupressiforme (H, Chil)
Llerasia macrocephala (H, Chil)
Novenia acaulis (H, Nov)
Oritrophium peruvianum (H, Chil)
Pteronia camphorata (H, Chil)
Madagaster madagascariensis (H, Mad)
Bellidiastrum michelii (B)
Bellis perennis (B)
Galatella linosyris (A)
Grangea maderaspatana (G)
Nidorella polycephala (G)
Tetramolopium vagans (P)
Olearia ramulosa (H, Ol)
Minuria integerrima (P)
Achnophora tatei (H, Cel)
Kalimeris pinnatifida (A)
Aster amellus (A)
Olearia arguta (H, Ol)
Remya kauaiensis (H, Rem)
Vittadinia sulcata (P)
Peripleura bicololor (P)
Tetramolopium pumilum (P)
Lagenophora pumila (L)
Kippistia suaedifolia (P)
Peripleura diffusa (P)
Minuria macrorhiza (P)
Myriactis humilis (L)
Keysseria maviensis (L)
Brachycome rigidula (Br)
Laestadia muscicola (H, Hin)
Laestadia pinifolia (H, Hin)
Hinterhubera imbricata (H, Hin)
Blakiella bartsiaefolia (H, Unp)
Parastrephia quadrangularis (H, Hin)
Parastrephia teretiuscula (H, Hin)
Diplostephium ericoides (H, Chil)
Floscaldasia hypsophila (H, Hin)
Westoniella chirripoensis (H, Hin)
Laennecia schiedeana (P)
Sommerfeltia spinulosa (P)
Aztecaster pyramidatus (H, Hin)
Myriactis panamensis (L)
Plagiocheilus bogotensis (G)
Archibaccharis androgyna (Bac)
Podocoma notobellidiastrum
Guynesomia scoparia (H, Unp)
Baccharis boliviensis (Bac)
Heterothalamus spartioides (Bac)
Doellingeria umbellata (U)
Eucephalus glabratus (U)
Egletes liebmannii (G)
Boltonia diffusa (Bo)
Chloracantha spinosa (Bo)
Canadanthus modestus (Sy)
Symphyotrichum vahlii (Sy)
Oreostemma alpigenum (U)
Eurybia wasatchensis (U)
Grindelia nana (M)
Pyrrocoma clementis (M)
Isocoma tenuisecta (M)
Rayjacksonia phyllocephala (M)
Machaeranthera tanacetifolia (M)
Gundlachia riskindii (S)
Ionactis linariifolia (U)
Chaetopappa effusa (Ch)
Croptilon divaricatum (Chr)
Noticastrum marginatum (Chr)
Erigeron bellidiastrum (C)
Erigeron subtrinervis (C)
Geissolepis suaedaefolia (As)
Petradoria pumila (S)
Eastwoodia elegans (S)
Chrysothamnus molestus (S)
Solidago sempervirens (S)
Pentachaeta exilis (Pe)
Ericameria resinosa (S)
F
1
2
outgroup
basal group
Southern Hemisphere grade
AUS
SA
North American clade

The initial heuristic search of the ETS data saving only 20 trees per replicate resulted in 13,700 trees 1627 steps long. Simple heuristic search using these asstarting trees found 242,000 trees of equal length (CI=0.408, RI=0.601, RC=0.245). In an attempt to sample the remaining tree space, the strict consensus tree of these trees was then employed as a reverse constraint in a subsequent heuristic search. Trees obtained in this last step were one step longer and congruent with the strict consensus tree. Bayesian analyses were run for $1.6x10^6$ generations sampling each 200^{th}-cycle. Resulting lnL values of the retained trees ranged from -8356.31 to -8396.27. The majority rule consensus tree is given in the figure 4.2.

Combined Data. The aligned matrix of the combined ETS/ITS data was 1159 bp long. The 5.8S subunit of the ITS part of the sequence were excluded from the analyses. A total of 250 (21.6%) characters were constant, 188 (16.2%) variable characters were parsimony-uninformative and 554 (47.8%) characters were parsimony informative. The partition homogeneity test (Farris et al. 1994) rejected the congruence of the two data sets (p=0.007). With the exclusion of outgroup taxa from the analyses, the congruence was not rejected (p=0.06). Visual inspection of the topologies inferred from the two data sets independently determined that, except for the placement of Felicinae, the two trees were congruent. Constraint analyses of the ETS data that forced Felicinae to have basal position within Astereae resulted in 2500 trees only one step longer than those recovered from the unconstrained analyses. Hence, the analyses of the combined data set were performed.

The initial heuristic search of the combined data saving 50 trees per replicate resulted in 92 trees 3536 steps long. Simple heuristic search using these trees as starting trees found 72 trees of equal length (CI=0.385, RI=0.571, RC=0.220). Strict consensus tree of these trees is

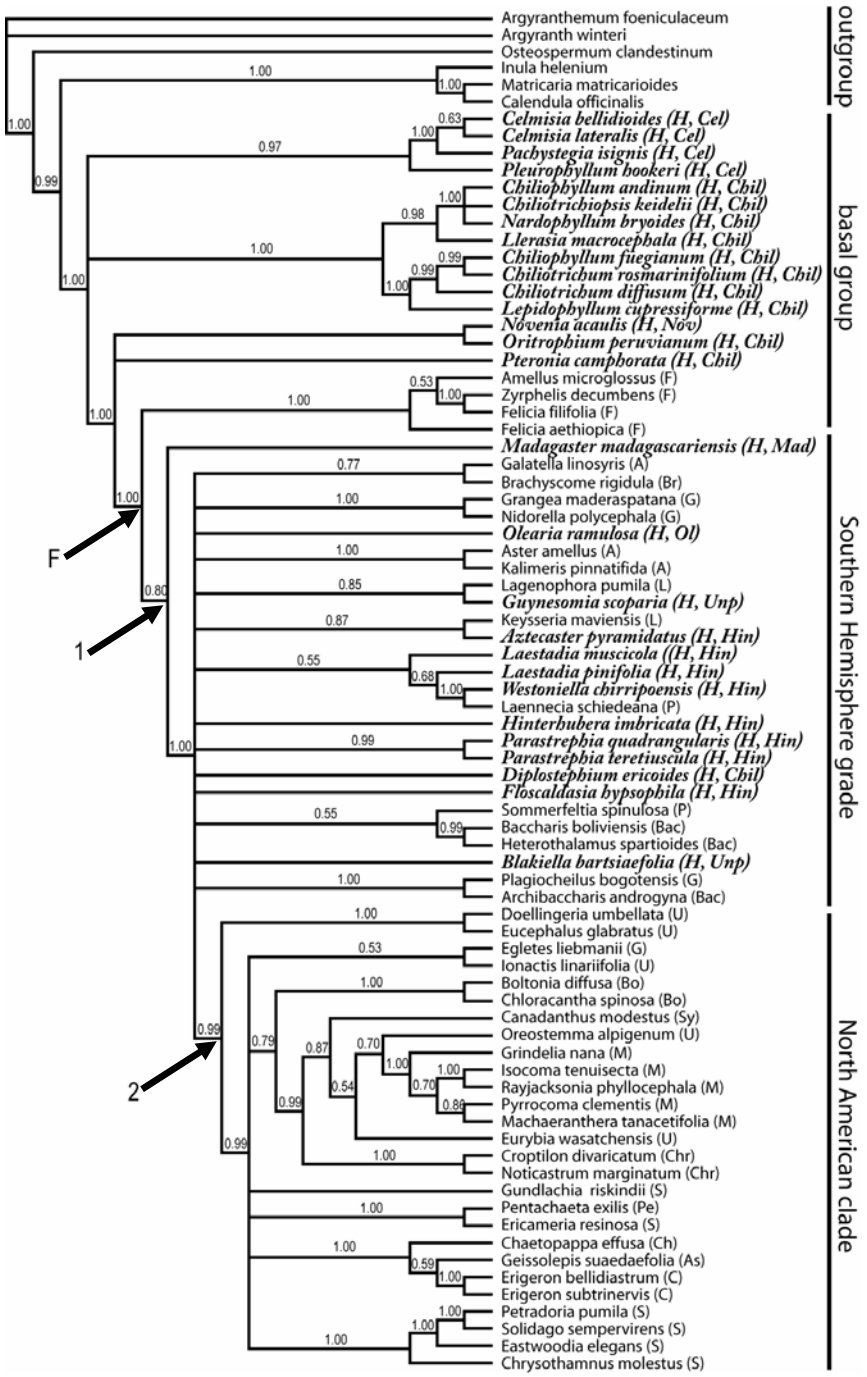


Figure 4.2. Phylogram inferred from Bayesian analyses based on ETS sequence data. Node 1 - distinguishes basal grade from the others; node 2 - supports the monophyly of the North American clade; node F - denotes Felicinae. Numbers above branches are posterior probabilities. Hinterhuberinae are in bold. Subtribe abbreviations and group affiliation given in parenthesis are as in figure 4.1.

given in figure 4.3. Bayesian analyses were run for $1x10^6$ generations sampling each 200^{th}-cycle. The lnL of the resulting trees varied from -18362.32 to -18395.89. The majority rule consensus of these trees is given in figure 4.4.

Phylogeny. Three major lineages in the tribe Astereae were identified in the Bayesian inference and MP analyses of the three data sets: basal group, South Hemisphere grade (SH) and North American clade (NA) (figs. 4.1-4.4). The exception was the strict consensus of the most parsimonious trees based on the ETS data set (data not shown) where these three major lineages were not recovered Instead the ingroup taxa formed a large polytomy with partially resolved relationships in the *Chiliotrichum, Celmisia* and the NA clades.

The basal group included *Chiliotrichum* (excluding *Diplostephium*), *Celmisia* and *Novenia* groups (*sensu* Nesom 1994), and Felicinae. Monophyletic *Chiliotrichum* and *Celmisia* clades grouped in a low supported lineage (PP=0.58, fig. 4.1; BS=69%, fig. 4.3) or their sister relationship was not recovered (figs. 4.2, 4.4). *Novenia* and *Oritrophium* were in a clade that was either sister to *Pteronia* (BS=62%, fig. 4.1, PP=0.92 PP=4.3, BS=51% fig. 4.4) or was part of a polytomy within the basal group (fig. 4.2).

The MP analyses of the ITS and combined data and Bayesian analyses of the ITS data supported basal position of the Felicinae within the Astereae, while the analyses of the ETS data and Bayesian analyses of the combined data placed Felicinae (node F) basal to the SH grade. Both positions of the Felicinae were highly supported (figs. 4.1-4.4; PP=1.00, BS=100%).

The SH grade included Grangeinae, Podocominae, Lagenophorinae, Asterinae, Baccharidinae and the remaining Hinterhuberinae. The relationship within SH grade was poorly resolved in all analyses. Somewhat better resolution of the SH grade was obtained in

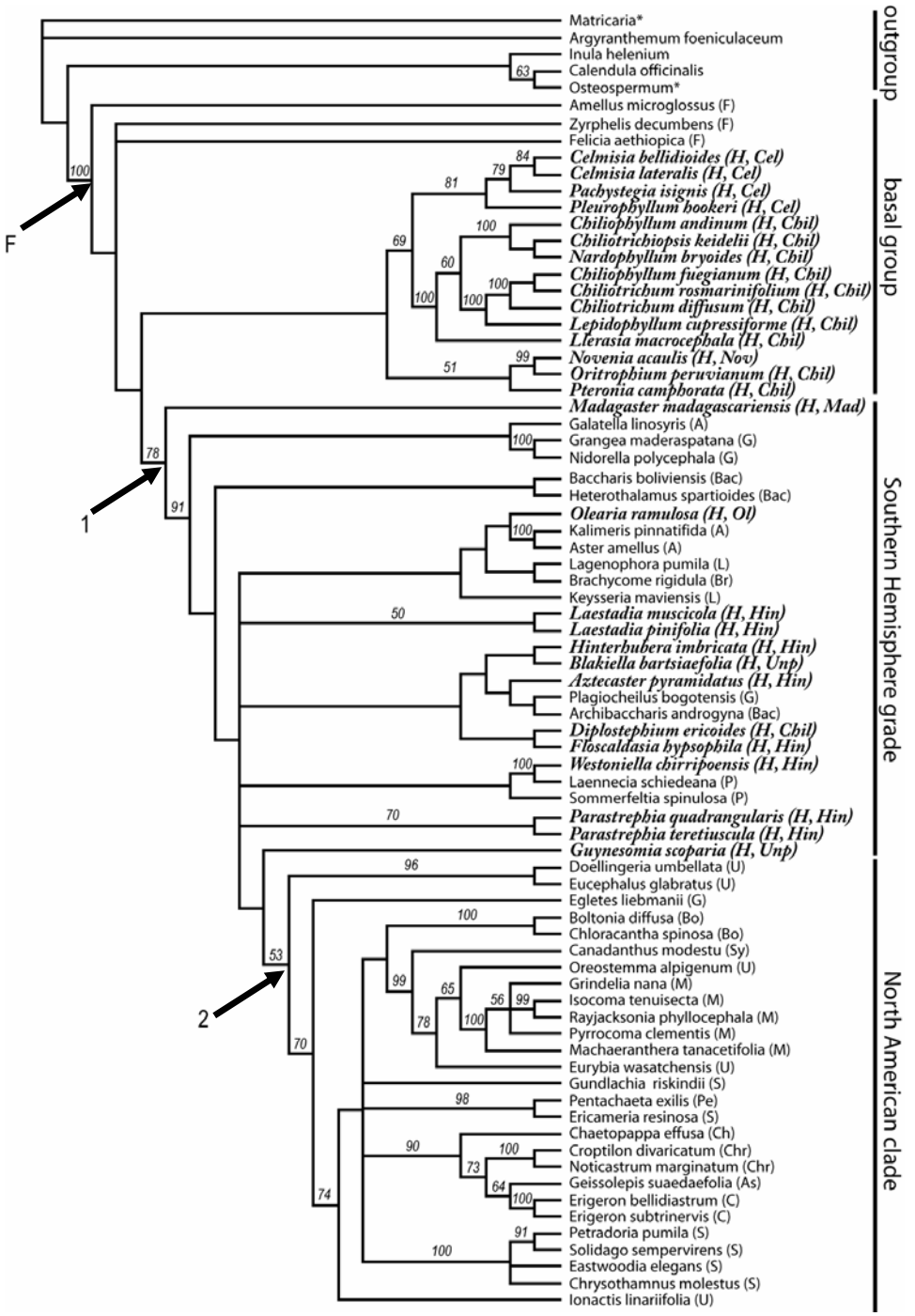


Figure 4.3. Strict consensus of 72 most parsimonious trees based on combined sequence data. Node 1 - distinguishes basal grade from the others; node 2 - supports the monophyly of the North American clade; node F - Felicinae. Bootstrap supports (<50% not shown) are numbers above branches. Hinterhuberinae are in bold. Subtribe abbreviations and group affiliation given in parenthesis are as in figure 4.1.

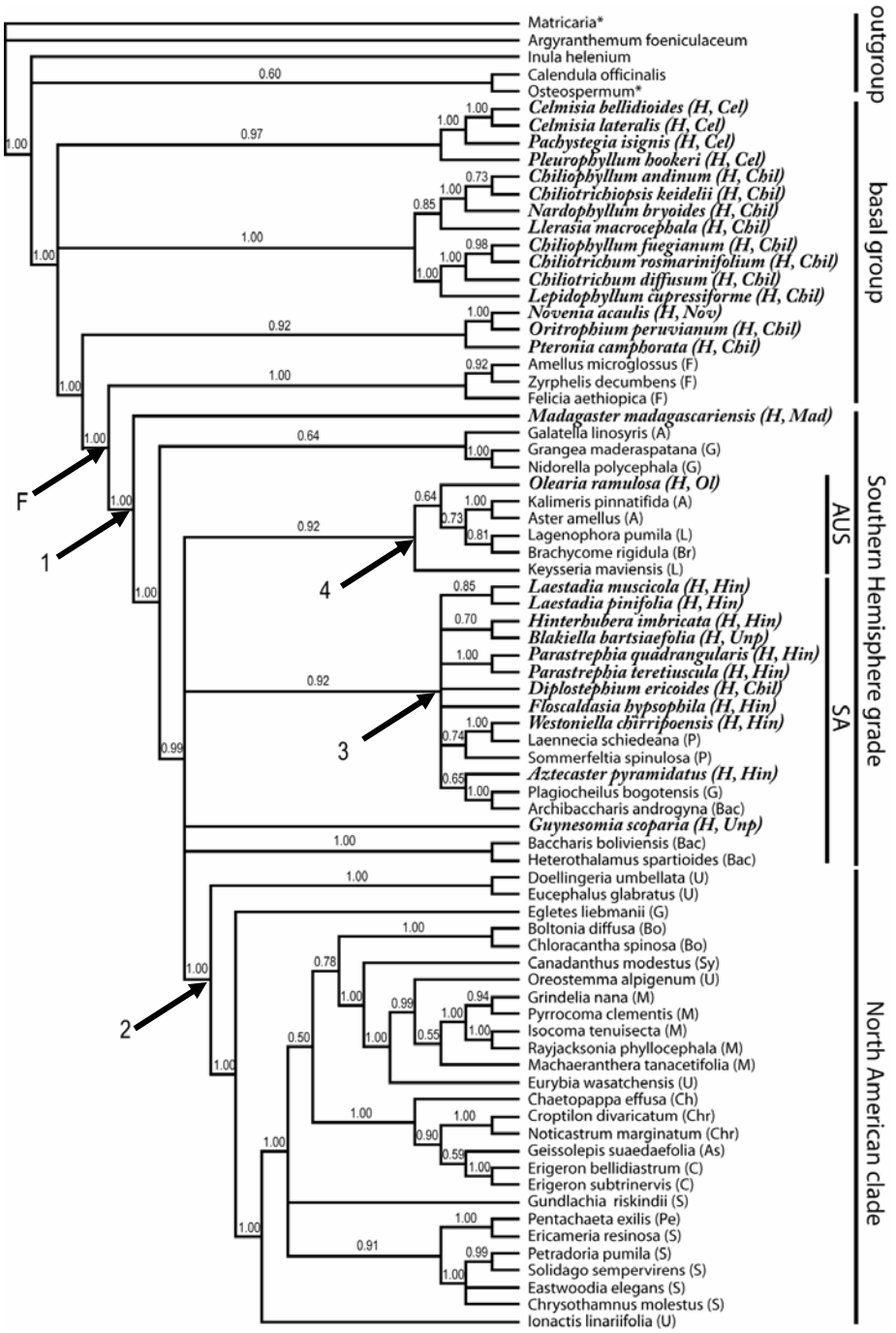


Figure 4.4. Phylogram inferred from Bayesian analyses based on combined data. Node 1-distinguishes basal grade from the others; node 2-supports the monophyly of the North American clade; node 3-denotes South American clade; node 4-denotes Austral clade; node F-Felicinae. Numbers above branches are posterior probabilities. Hinterhuberinae are in bold. Subtribe abbreviations and group affiliation given in parenthesis are as in figure 4.1.

the ITS and combined data analyses (figs. 4.1 and 4.4). Successive approximations weighting decreased the level of homoplasy in the ITS tree (value of CI increased from 0.334 to 0.565). The strict consensus of the trees recovered in three runs was highly congruent with the MrBayes inferred topology, thus resulting in resolved relationships within SH grade. All the subtribes appeared polyphyletic. *Madagaster* was basal in the SH grade (PP>0.80, BS>81%) in all phylogenies. Taxa representing Grangeinae were in the SH grade (*Grangea, Nidorella,* and *Plagiocheilus*) and also in the NA clade (*Egletes*). *Kalimeris* and *Aster* both typically treated as Asterinae were not related to its supposed congener *Galatella*. Instead the later was close to Bellidinae (PP= 0.98, BS=71%, fig. 4.1). The ITS and combined Bayesian inferred topologies divided Podocominae, Lagenophorinae and Hinterhuberinae in two main polytomies. The clade named Austral (figs. 4.1 and 4.4) contained taxa from Hinterhuberinae, Podocominae, Lagenophorinae and Brachycominae distributed in Australia, New Zealand and Hawaii. The other polytomy, labeled South American (figs. 4.1 and 4.4) included taxa from Hinterhuberinae, Podocominae, Lagenophorinae and Baccharidinae distributed in South and Central America.

The North American clade was recovered in all analyses with moderate to high support (PP>0.99, BS=72%). This clade included taxa belonging to predominantly North American subtribes, Astranthiinae, Boltoniinae, Chrysopsidinae, Solidagininae, Machaerantherinae, Chaetopappinae, Penthachaetinae, and Symphyotrichinae, with the exception of *Egletes* (Grangeinae).

Biogeography. Reconstruction of the ancestral area on one of the trees selected randomly from Bayesian analyses of ITS and combined data sets using MacClade resolved southern Africa, South America and New Zealand as possible centers of origin for Astereae

(fig. 4.5-4.7). In addition, the ITS data indicated Eurasia and North America also as possible centers of origin (fig. 4.6). Their selection as centers of origin was possibly influenced by the outgroup selection since they are all from Eurasia or North America. Origination of Astereae in Eurasia or North America is unlikely because the greatest diversity of putatively primitive members ("basal group") is in South America, southern Africa and Australasia. However, Eurasia cannot be eliminated from consideration since it holds many Astereae that have not yet been sampled.

South America, New Zealand or southern Africa were resolved as possible centers of origin for the *Chiliotrichum* and *Celmisia* groups based on the ITS data alone (figs. 4.5, 4.6). *Hinterhubera* group, however, as part of the Southern Hemisphere grade appears to have evolved from the elements in South America based on the both ITS and combined data tree reconstructions (figs. 4.5-4.7).

DISCUSSION

Position of Felicinae. The topologies were consistent with those obtained by Noyes and Rieseberg (1999) in resolving three main lineages: basal group, Southern Hemisphere grade and North American clade. Their analyses, rooted at Anthemideae, Calenduleae, Gnaphalieae and Inuleae, resolved Felicinae basal to the remaining Astereae. Molecular analyses of *Olearia* (Cross et al. 2002) and *Nannoglottis* (Liu et al. 2002) based on the ITS data, and studies of *Printzia* based on matK sequence data (Bayer and Cross 2002), placed Felicinae basal to the SH grade, thus having a derived position relative to the *Chiliotrichum* and *Celmisia* clades. Constrained analyses of the ETS data that forced basal placement of

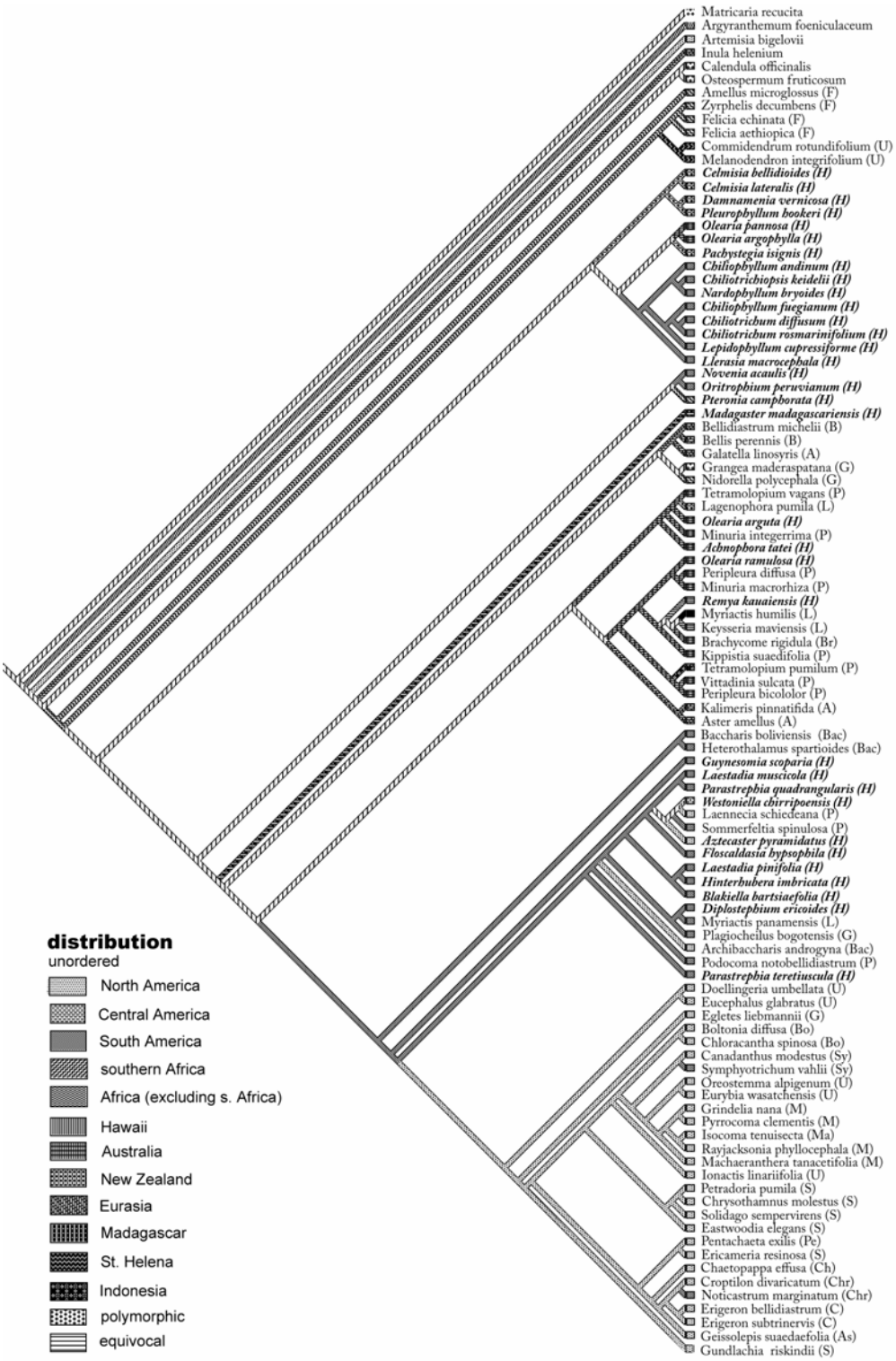


Figure 4.5. Biogeographical reconstruction on randomly selected tree from Bayesian analyses of ITS data. Hinterhuberinae are in bold. Subtribe abbreviations are as in figure 4.1.

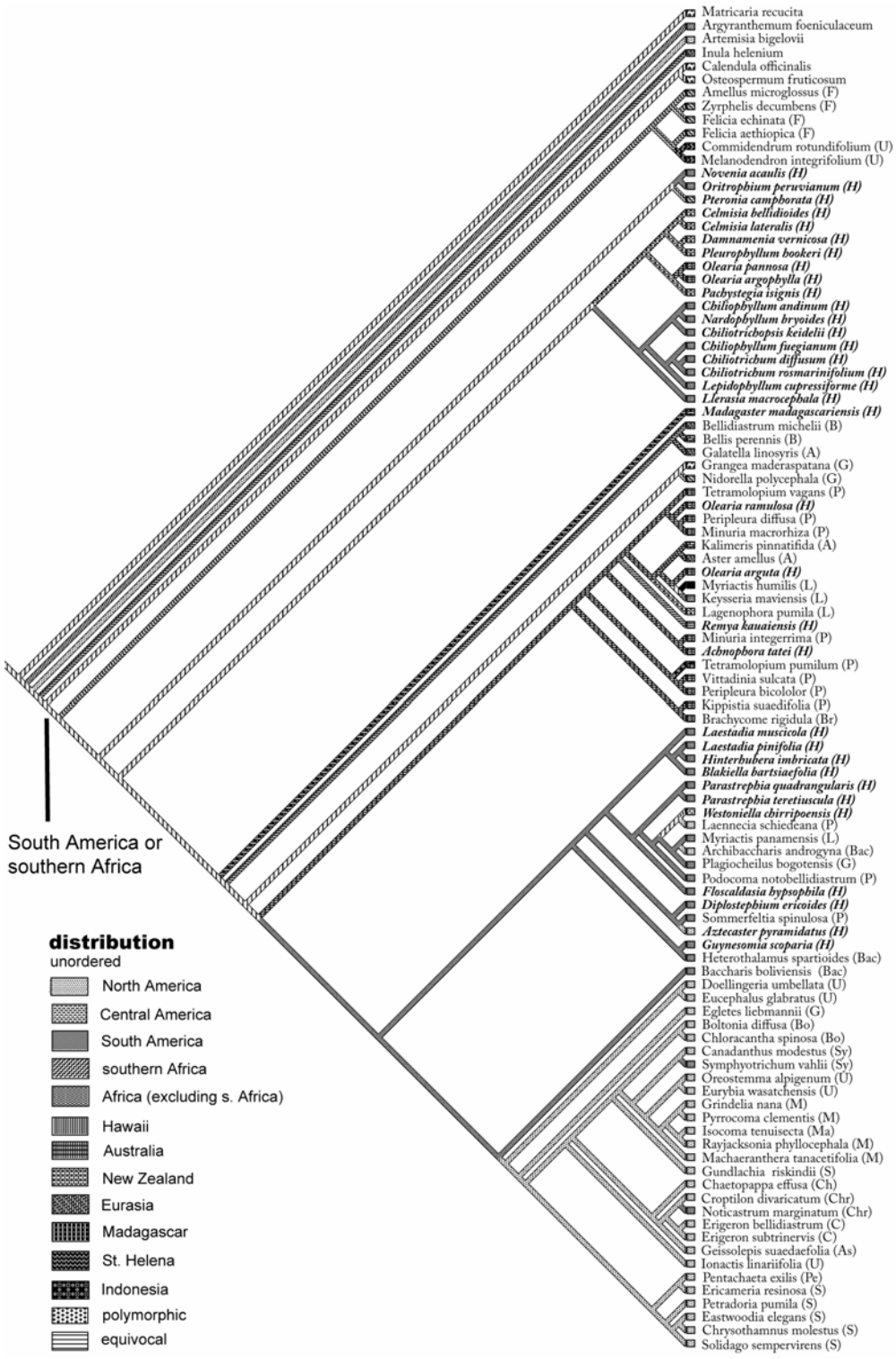


Figure 4.6. Biogeographical reconstruction on randomly selected tree from Bayesian analyses of ITS data. Hinterhuberinae are in bold. Subtribe abbreviations are as in figure 4.1.

Figure 4.7. Biogeographical reconstruction on randomly selected tree from Bayesian analyses of combined data. Hinterhuberinae are in bold. Subtribe abbreviations are as in figure 4.1. Note the derived position of Felicinae.

Felicinae resulted in trees that were only one step longer than the unconstrained topologies (data not shown). Therefore, at this level of sampling it is difficult to conclude what is basal within Astereae. The ITS data and parsimony analyses of combined data supported Felicinae as basal, while ETS and Bayesian analyses of the combined data supported the *Chliotrichum*-*Celmisia* clade in this position.

Monophyly of Hinterhuberinae. As discussed previously (see chapters 2 and 3) subtribe Hinterhuberinae *sensu* Nesom (1994) is polyphyletic. The *Chiliotrichum*, *Celmisia* and *Novenia* groups were basal in the tribe Astereae. These are woody species (shrubs and subshrubs) adapted to high elevation ecosystems of the South America, Australia, New Zealand, and the sub-Antarctic islands. The only herbaceous genera are *Oritrophium* and *Novenia* from the Andean páramos. One genus, *Pteronia*, is distributed in southern Africa. Detailed discussion of the basal taxa is given in chapter 3.

The SH grade included *Madagaster*, *Remya*, *Hinterhubera* and part of the *Olearia* group. Included in the *Madagaster* group (*sensu* Nesom 1994) are three genera, *Madagaster*, *Rochonia* and *Mairia*. Here the group was represented by *Madagaster*. The former two are distributed in Madagascar, and the later in Africa. *Madagaster* are small trees or shrubs with large white-rayed radiate capitula and epaleate receptacles. Humbert (1932) identified its five species as *Aster*, but later pointed out their similarity to *Diplostephium* and *Olearia* (Humbert 1960). Nesom (1993c) transferred the white-rayed Madagascan species into a new genus *Madagaster* and placed this genus in the Hinterhuberinae based on its shrubby habit, coriaceous leaves, dense tomentum and multinerved cypselae. Molecular analyses however,

isolate *Madagaster* basal in the SH grade suggesting a distant relationship to the Hinterhuberinae.

The *Remya* and *Hinterhubera* groups (*sensu* Nesom 1994) were not related to each other. Several species from Lagenophorinae and Podocominae were closer to *Hinterhubera* group than to the remaining genera from their previously assigned subtribes. More detailed discussion related to this clade is presented in chapter 2. Suggested close relationships of *Remya* with *Olearia* (Wagner and Herbst 1987, Nesom 1994) was unsupported in the present analyses. *Achnophora,* a genus from the Kangaroo Islands, Australia, had an unknown position within Astereae (Zhang and Bremer 1993, Bremer 1994) or was tentatively placed within Hinterhuberinae (Nesom 1994). Molecular analyses did not support its relationship to *Olearia* (Nesom 1994) or any other genus from Hinterhuberinae *sensu lato*.

EVOLUTION OF MORPHOLOGICAL CHARACTERS

Habit. Woody (Grau 1977, Noyes and Rieseberg 1999, Cross et al. 2002) and herbaceous habits (Bremer 1994) have variously been suggested as plesiomorphic for Astereae. Herbaceous forms were also hypothesized to be ancestral for the entire family (Stuessy et al. 1996). In the ITS topologies, fifteen out of twenty four taxa in the basal clade are woody (trees, shrubs, or subshrubs, fig. 4.8). Optimization of the character on one of the MrBayes inferred trees based on the ITS data resolved woody habit as ancestral, while herbaceous habit appeared in three lineages, Felicinae, *Oritrophium-Novenia* and *Celmisia*. The SH grade contains taxa with both shrubby and perennial herbaceous forms. The basal *Madagaster* includes trees and shrubs. No coherent tendency in the evolution of the forms could be observed within the SH grade, probably because of its low resolution. In at least one

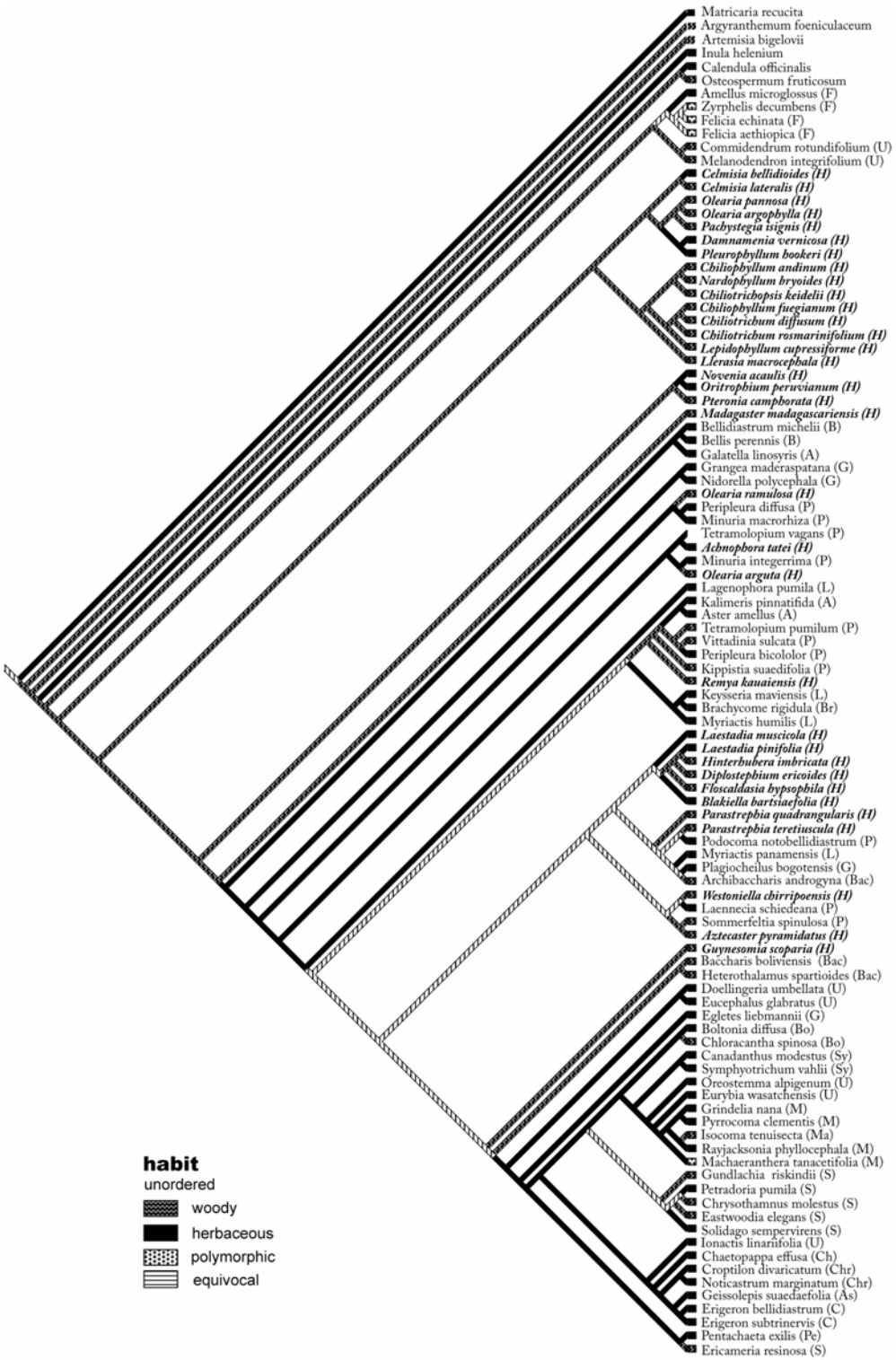


Figure 4.8. Optimization of habit on one of the trees obtained from Bayesian analyses of ITS data. Hinterhuberinae are in bold. Subtribe abbreviations are as in figure 4.1.

case shrubby species were sister to herbaceous species (*Westoniella-Laennecia* clade, PP=1.00, BS=99%, fig. 4.1). The NA clade predominantly consists of herbaceous plants. In some genera from NA clade the woody habit may represent a secondary reversion from herbaceous to woody form (Stebbins 1977).

Receptacular Paleae and Staminate Disk Florets. One of the characters delimiting the South American *Chiliotrichum* from the *Hinterhubera* group is the presence of receptacular paleae in the former. This character is not uniform throughout the *Chiliotrichum* group, as they are absent in *Lepidophyllum*, *Llerasia* has only 1-2 paleae, some *Nardophyllum* species lack paleae, and in *Chiliophyllum*, *Chiliotrichum* and *Chiliotrichiopsis* receptacles are entirely or partially paleaceous. Character optimization in MacClade on one of the MrBayes inferred trees based on the ITS data supported the independent evolution of receptacular paleae in *Amellus* (Felicinae), the *Chiliotrichum* clade, *Achnophora, Diplostephium* (Hinterhuberinae), *Heterothalamus* (Baccharidinae), and in two North American taxa (fig. 4.9). Several parallel gains are regarded as more parsimonious than multiple losses from the paleate condition. Hence, receptacular paleae can be considered a novelty within the tribe rather than plesiomorphic (Keil and Stuessy 1981, Zhang and Bremer 1993, Eldenäs et al. 1998). *Lepidophyllum* from the monophyletic *Chiliotrichum* clade (excluding *Pteronia, Oritrophium* and *Diplostephium*) experienced either secondary loss of paleae or it was the only genus with the preserved plesiomorphic epaleate character.

Lepidophyllum is also the only genus in the *Chiliotrichum-Celmisia* clade that has functionally staminate disk florets. Functional bisexual florets are considered plesiomorphic and are found in the *Chiliotrichum* and *Celmisia* clades (fig. 4.10). Functionally staminate disk florets have evolved several times in the Asteraceae, and are known from many genera in

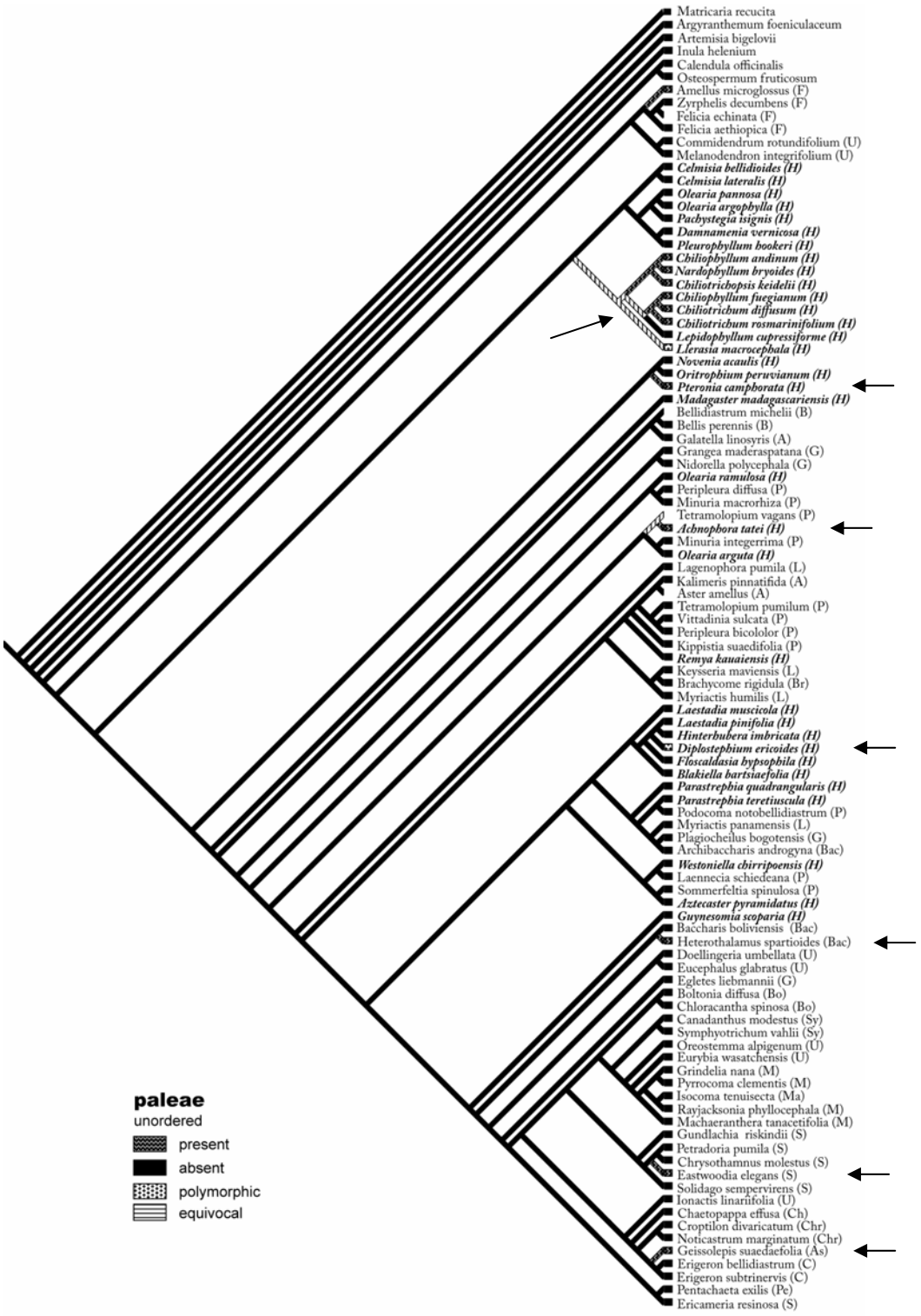


Figure 4.9. Optimization of presence of receptacular paleae on one of the trees obtained from Bayesian analyses of ITS data. Hinterhuberinae are in bold. Subtribe abbreviations are as in figure 4.1. Arrows indicate species with receptacular paleae.

the tribe Astereae (Bremer 1994). Optimization of the disk floret function in MacClade on one of the MrBayes inferred trees based on the ITS data supported the independent evolution of staminate disk florets in several lineages, Felicinae, *Novenia-Oritrophium* clade, SH grade and NA clade (fig. 4.10). In the SH grade, the character evolved in Podocominae (*Tetramolopium*, *Minuria*), Lagenophorinae (*Myriactis*) and Hinterhuberinae (*Achnophora*, Remya and *Hinterhubera* group) independently. Disk florets in *Remya* are of two types, the outer series consists of pistillate or neuter florets and the inner series are staminate. Most species in *Diplostephium* possess bisexual disk florets, but in some they are staminate. These changes in the function of disk florets may be result of mutations within a simple genetic system (Turner 1978).

Ligule of the Ray Floret Corolla. Typical pistillate floret corollas in the Asteraceae capitulum consist of a tube and an elongated ligule. Stuessy et al. (1996) suggested that the ligulate corolla has developed rapidly from a primitive, tubular, 5-lobed, actinomorphic corolla. Differentiation of the ligule was probably associated with adaptation to insect pollination (Lane 1996). Therefore, any reduction in the ligule may be considered to be result of secondary reversal. In the tribe Astereae, reduction of ligule and complete loss of ligulate pistillate florets evolved independently in several groups. Optimization of the ligule of the ray floret corolla in MacClade on one of the MrBayes inferred trees based on the ITS data supported the independent reduction of ligule and complete loss of ligulate pistillate florets in Grangeinae, Brachycominae, Baccharidinae and *Hinterhubera*, *Remya* and *Novenia* groups from Hinterhuberinae (fig. 4.11).

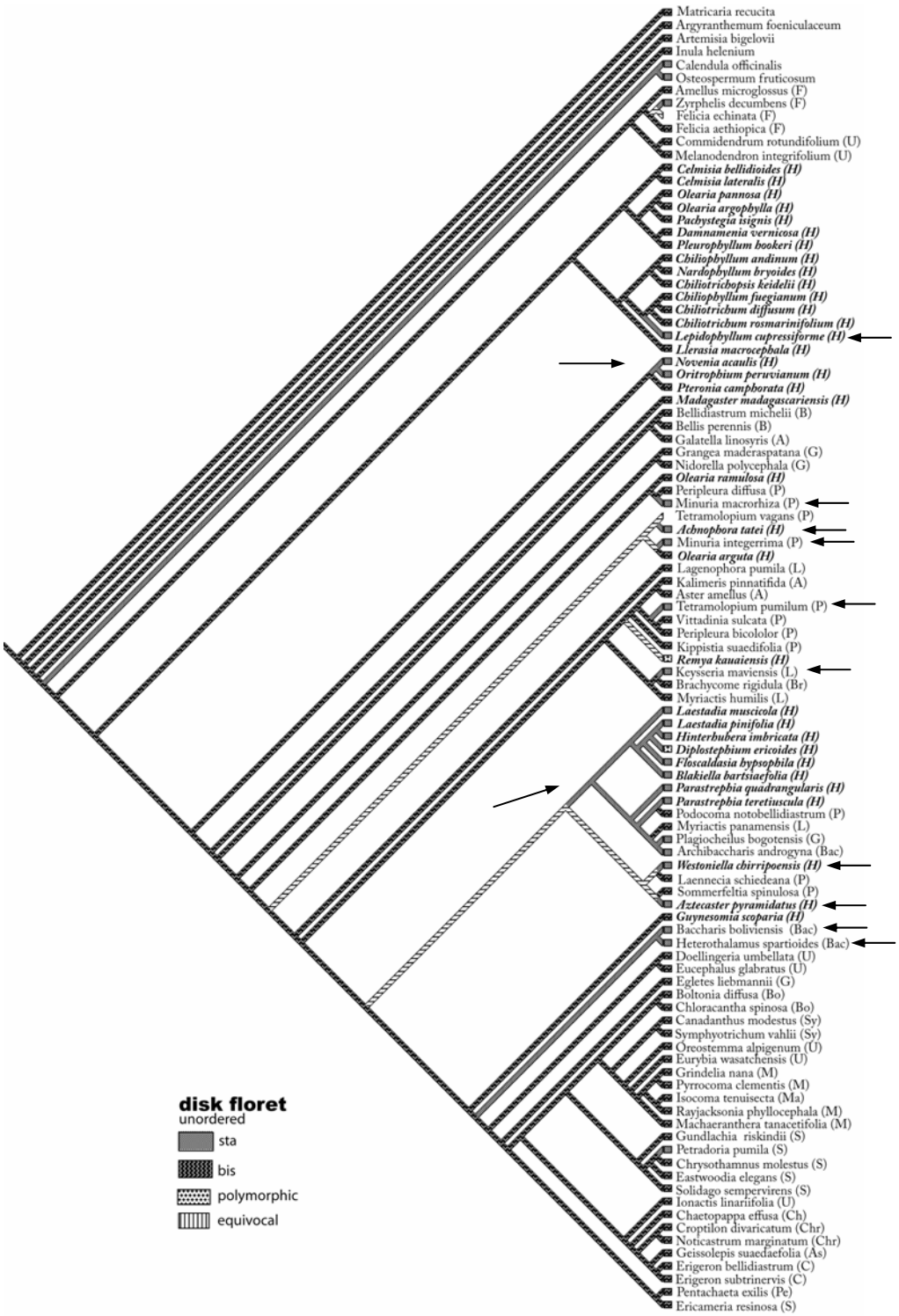


Figure 4.10. Optimization of disk florets on one of the trees obtained from Bayesian analyses of ITS data. Hinterhuberinae are in bold. Subtribe abbreviations are as in figure 4.1. Arrows indicate staminate disk florets.

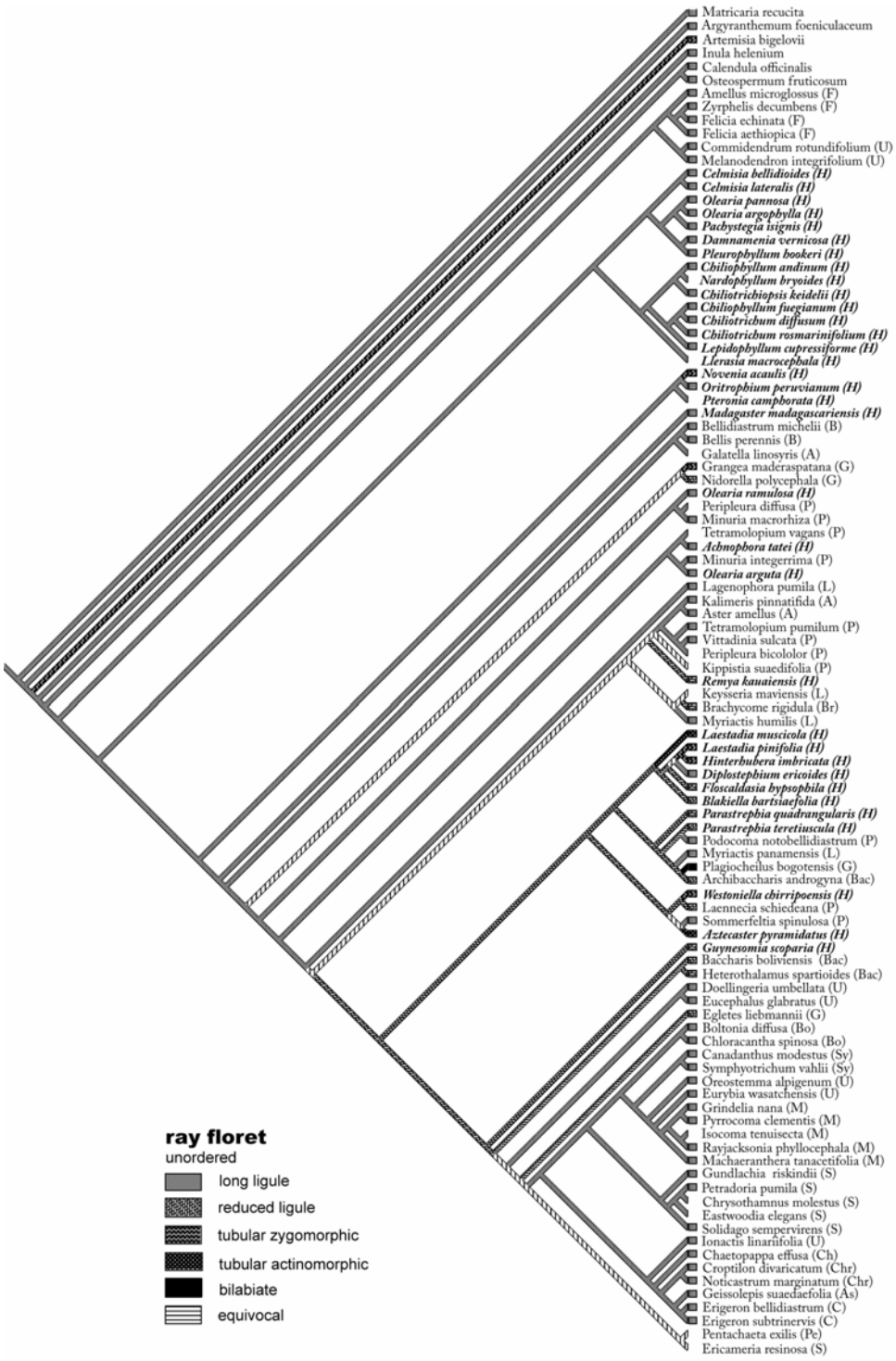


Figure 4.11. Optimization of the morphology of pistillate ray florets on one of the trees obtained from Bayesian analyses of ITS sequence data. Hinterhuberinae are in bold. Subtribe abbreviations are as in figure 4.1. Ligule shape in the species with discoid capitula was coded as missing.

Well developed ligules characterize the basal group. Two genera of Hinterhuberinae in the basal group, *Llerasia* and *Nardophyllum* have discoid capitula, and *Novenia* has zygomorphic, tubular ray florets with very short ligules. The SH grade shows more variation in ligular morphology. The *Hinterhubera* group in general includes genera with reduced ligules or its pistillate corollas are tubular with long asymmetric lobes (e.g. *Hinterhubera*). *Laestadia* exhibits an unusual form characterized by tubular, actinomorphic corollas with abruptly widened limb. In dioecious *Aztecaster,* however, the corollas of its pistillate capitula are actinomorphic tubular and narrow. The minute pistillate corollas in *Plagiocheilus* are bilabiate. The adaptive advantages of these modifications in the morphology of the ray corollas is unclear. Nothing is known about the ecology and pollination mechanisms of these plants, and their significance can only be speculative. Similar to *Hinterhubera* group and *Novenia-Oritrophium* clade, *Espeletia* (Heliantheae, Asteraceae) is also found above 4000 m altitude in Andes where the number of available pollinators decreases radically. Berry and Calvo (1989) found that reduced ligules present in some species of *Espeletia* were correlated with wind pollination which they considered derived. However, these wind pollinated plants of *Espeletia* are tall with hanging capitula displayed high above the ground where wind would be an obvious, effective pollination agent. Species in the *Hinterhubera* group and *Novenia*, however, are small shrubs or low growing herbaceous perennials, about 10-30 cm tall usually forming dense mats. Only a few species reach a height of more than 1 m and none has hanging capitula. In the mat-forming *Novenia,* capitula are in dense sessile clusters between the leaves, and in other cushion forming genera capitula are either on the branch tips (*Westoniella*) or on the short stalks (*Floscaldasia, Laestadia*). As discussed by Swenson and Bremer (1997), the sun radiation increases the temperature of these cushions. The

temperatures may be several degrees higher than in the surrounding environment, producing an attractive feature for small beetles that swarm over them. Therefore, capitula that are close to the ground have higher probability of being visited by these possible pollinators. In that case, the presence of ligules may be redundant and unnecessary investment.

TAXONOMIC IMPLICATIONS AND INFRATRIBAL RELATIONSHIP

Molecular analyses did not support current delimitation of Hinterhuberinae (Nesom 1994, Nesom and Robinson 2006). Some members were closer to other subtribes than to each other. *Chiliotrichum*, *Celmisia* and *Novenia-Oritrophium* clades were in the basal group close to Felicinae (fig. 4.1). *Remya*, *Olearia* in part, *Madagaster* and *Hinterhubera* clades were in the SH grade with other Southern Hemisphere subtribes. *Novenia-Oritrophium* and *Madagaster* had somewhat isolated positions in the basal group and at the base of the SH grade, respectively. Morphological characters that separate *Chiliotrichum-Celmisia* clades from Hinterhuberinae in the SH grade include well developed ray corolla ligules, bisexual disk florets (except *Lepidophyllum* that has staminate disk florets), high levels of polyploidy (6n-12n), and presence of receptacular paleae which are absent in *Lepidophyllum*. In addition, there is a geographic and ecological separation between genera from *Chiliotrichum* and *Hinterhubera* groups. Genera from the *Chiliotrichum* clade are restricted to Patagonia and the puna region of the Andean Cordillera. *Llerasia* is the only genus distributed in the northern parts of South America, but it is restricted to the uppermost montane forest of the Andes present below the páramo zone. The *Hinterhubera* group inhabits páramos of the Andean Cordillera.

Based on molecular analyses and supported by certain morphological characters mentioned above, subtribe Hinterhuberinae is not monophyletic and needs to be redefined.

The *Chiliotrichum* and *Celmisia* clades should be excluded from the subtribe defining a new group that is closer to the ancestral stock of Astereae. Molecular analyses suggested including *Novenia-Oritrophium* clade and the genus *Pteronia* within this new group, but this could not be supported by morphological characters. Further detailed morphological analyses are needed to evaluate these genera and find possible synapomorphies with the *Chiliotrichum* and *Celmisia* clades.

Molecular data did not support inclusion of *Madagaster* in the subtribe HInterhuberinae. Unfortunately no sequence data were available for *Rochonia*, another related Madagascan genus with similar morphology. Phylogenetic analyses corroborated Cuatrecasas' (1986) treatment of the subtribe and Nesom's (1994) delimitation of the informal *Hinterhubera* group with three possible additions, *Blakiella*, *Diplostephium* and *Guynesomia*. *Blakiella* is not related to Podocominae, but to *Hinterhubera. Diplostephium* was closer to the *Hinterhubera* group than to the *Chiliotrichum* group. *Guynesomia*, although related to the *Hinterhubera* group, was not strictly part of it. The new, redefined Hinterhuberinae will probably include *Hinterhubera* group (*sensu* Nesom 1994), *Diplostephium*, *Guynesomia* and *Blakiella*. However, caution should be exercised in delimiting this reduced subtribe Hinterhuberinae because its monophyly received low branch support or was not recovered in the analyses of the ETS data and in the MP analyses of combined data. Placement of *Laennecia*, *Podocoma notobellidiastrum* and *Sommerfeltia* (Podocominae) close to genera from *Hinterhubera* group questions the current generic delimitation and Southern Hemisphere subtribes classification. This research included only small number of taxa from various Southern Hemisphere subtribes. More extensive sampling

of genera from Lagenophorinae, Podocominae, Grangeinae and Asterinae is definitely in order.

BIOGEOGRAPHY

Based on morphological, molecular, biogeographic and fossil data Asteraceae and its major tribes have their origin in the ancient Gondwanaland area: Africa, South America, and Australia (Bremer 1992, 1994, Bremer and Gustafsson 1997). In particular, it is suggested that the origin is in southern South America (Stuessy et al. 1996) or northern South America or northern Andes (Turner 1977, Raven and Axelrod 1974). The tribe Astereae was suggested by several authors to have arisen in the Southern Hemisphere (Zhang and Bremer 1993, Bremer 1994, Nesom 1994, Noyes and Rieseberg 1999), probably in upper Cretaceous or later, at the time when the distribution between the continents was more probable.

South America, southern Africa and New Zealand were the three possible centers of origin for the tribe Astereae (fig. 4.9-4.11) suggested by the ITS and combined data. Present distribution of taxa from the tribe Astereae in South America and southern Africa was hypothesized to be result of drifting continents in upper Cretaceous (Turner 1977, Nesom 1994). Stuessy et al. (1996) argued that this hypothesis seemed unlikely because no fossil collections from the family Asteraceae are known prior to Oligocene. Even then, it is possible that the dispersion between the two continents happened through the stepping-stones (small islands) that were present during the Oligocene as the level of the ocean was low (Tarling 1980). The cypselae of Astereae typically have pappus bristles that are considered to be modified calyx (Cronquist 1981) adapted in many cases to wind dispersal, but it also plays other adaptive roles, such as protection against herbivorous insects (Stuessy and Graver 1996). In some species, such as Hinterhuberinae *sensu* Nesom (1994), cypselae are covered

by glandular trichomes what makes the fruits sticky and presumably easy to attach to birds feathers. Distribution into or from Australia and New Zealand was probably through Antarctica prior the middle Eocene when Australia was still attached to Antarctica.

Chiliotrichum and *Celmisia* clades may have originated either in New Zealand, South America or southern Africa (figs. 4.9-4.11) Dispersal between New Zealand and South America probably occurred through Antarctica that functioned as a bridge between these continents. These clades possibly originated in Antarctica, which had cool temperate climate until the late Eocene that certainly would support the existence of ancestral stock that eventually spread into New Zealand, Australia, and South America. The importance of Antarctica as a dispersal route was discussed by Wardle (1978) and by Given and Gray (1986).

The *Hinterhubera* group was resolved to have evolved within South America (figs. 4.9-4.11). Due to the young age of the present day páramo zone (Simpson 1975), this group may have evolved recently from the autochthonous elements from lower altitudes with the uplift of the Andes. It seems likely that the genera spread from the southern and central Andes as proposed by neotropical páramo vegetation studies (Cleef 1978), during the ice age when the páramo zone became wider covering lower elevations, thus allowing species to spread between different "islands" of mountain peaks.

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CHAPTER 5 PRELIMINARY RESULTS ON THE PHYLOGENY OF HINTERHUBERINAE INFERRED FROM THE 5' trnK/matK INTERGENIC SPACER OF cpDNA SEQUENCE DATA

Chloroplast DNA has been used as either the primary source of data or has been combined with the ITS sequence data of the nrDNA for inferring plant phylogenies at various taxonomic levels (Shaw et al. 2005). The matK coding region of the cpDNA is a maturase encoding gene located at the intron of the transfer RNA gene for the lysine (trnK). The trnK intron is of the type II introns, and it is about 2500 bp long, of which about 1500 bp encode the matK (Hilu and Liang 1997; fig. 5.1). Compared to other chloroplast regions, the matK coding region is fast evolving and thus suitable for phylogenetic analyses at the intrafamilial level. It has 27% higher percent of potentially informative sites than the trnL and trnF chloroplast spacer regions (Bayer et al. 2002, Samuel et al. 2003), and it is two times more variable than the rbcL (Steele and Vigalys 1994). The region was utilized in phylogenetic studies of different plant groups (Johnson and Soltis 1994, Steele and Vigalys 1994, Bayer et al. 2002, Paun et al. 2005, Wojciechowski 2005). In Asteraceae, matK sequences were used independently or combined with other cpDNA or nrDNA sequence data in reconstructing generic or higher-level phylogenies (Garcia-Jacas et al. 2001, Bayer et al. 2002, Bayer and Cross 2002, Chan et al. 2002, Samuel et al. 2003, Crawford and Mort 2005).

The 5' portion of the matK coding region is phylogenetically more informative than the 3' end (Bayer et al. 2002). In the present study, the 5' trnK/matK intergenic region was utilized to assess phylogeny in the subtribe Hinterhuberinae. The region will be designated as matK in the remaining text.

MATERIALS AND METHODS

Taxon Sampling. The sampling of taxa was designed to include at least one species from each genus sampled in the analyses of the nrDNA (chapter 4). The amplification and sequencing of about 2000 bp long matK region was difficult or unsuccessful from the DNA obtained from older herbarium material. Therefore the final data set included only 25 taxa (tab. 5.1). Additional matK sequences of 9 taxa were obtained from Bayer and Cross (2003) and one sequence from Wagstaff and Breitwieser (2004). Based on the previous molecular analyses that resolved Anthemideae and Calenduleae sister to Astereae (Wagstaff and Breitwieser 2002, Funk et al. 2005), and the availability of the sequences in GenBank, four species from these tribes were chosen for the outgroup. The ETS/ITS and matK sequences for *Osteospermum* and *Matricaria* were composed from two different species in the analysis of the combined dataset, as noted in the table 5.1.

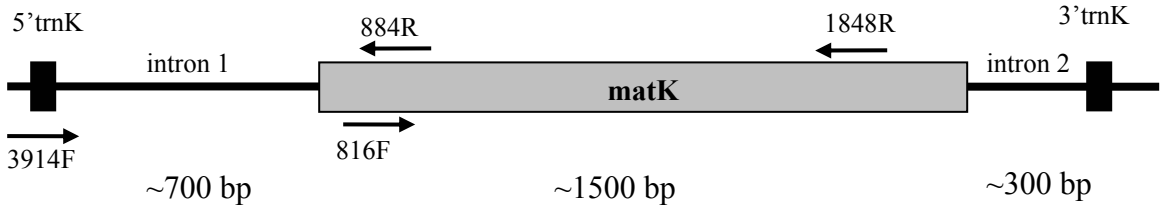

Figure 5.1. Schematic presentation of the trnK intron and the matK coding region of the cpDNA. Relative length of the coding region and the introns, and relative position of the PCR amplification and sequencing primer pairs used in this study are given. Arrows indicate the direction of strand synthesis. Boxed areas represent coding regions.

DNA Extraction, Amplification and Sequencing. Total DNA was extracted following protocols outlined in chapter 2. Amplification of the matK was done using two sets of primers: 3914F (Johnson and Soltis 1994) and 884R (Panero and Crozier

Table 5.1. List of taxa, collection information and GenBank numbers. References for sequences obtained from other sources are given.

SPECIES LIST	LOCALITY	COLLECTOR (HERBARIUM)	GENBANK ITS #	GENBANK ETS#	GENBANK matK	REFERENCE
Outgroupa taxa						
ANTHEMIDEAE						
Artemisia tridentata Nutt.	USA: Colorado	Columbus 3874 (RSA)			AF456776	Bayer and Cross 2003
**Matricaria recucita* L.		MATI2 (J. L. Hudson Seedsman, Redwood City, CA, USA)				Francisco-Ortega et al. 1997
ITS1			AF155271			
ITS2		"	AF155308			"
**Matricaria matricarioides* (Less.) Porter		Bayer AB-95005 (CANB)		AF319724		Bayer et al. 2002
**Matricaria matricarioides* (Less.) Porter	Canada: Alberta	Bayer AB-95005 (CANB)			AF151481	Bayer and Cross 2003
CALENDULEAE						
Calendula officinalis L.	cult. New Zealand, Christchurch	Breitwieser 2129 (CHR)	AF422114			Wagstaff et Breitwieser 2002
Calendula officinalis L.	South Africa: Commercial source	Bayer GH-95009 (CANB)		AF319684		Bayer et al. 2002.
Calendula officinalis L.	South Africa: Commercial source	Bayer GH-95009 (CANB)			AF151446	Bayer and Cross 2003
**Osteospermum clandestinum* (Less.) Norl.	Australia: Western Australia	Bayer WA-94070 (CANB)		AF319733		Bayer et al. 2002
**Osteospermum clandestinum* (Less.) Norl.	Australia: Western Australia	Bayer WA-94070 (CANB)			AF151488	Bayer and Cross 2003
**Osteospermum fruticosum* (L.) Norl.	cult. New Zealand, Christchurch	Breitwieser 2131 (CHR)	AF422131			Wagstaff & Breitwieser 2002

table 5.1 continued

Ingroup taxa						
ASTEREAE						
Subtribe Asterinae						
Galatella linosyris Reichb.	Austria:	Seiller s.n (F)	DQ478987	DQ479043	x	
Subtribe Baccharidinae						
Baccharis boliviensis (Wedd) Cabrera	Argentina: San Louis: Coronel Pringles; 1532 m alt.	R. H. Fortunato 5518 (USA)	DQ478992	DQ479048		
Heterothalamus spartioides Hook. & Arn.	Argentina: Rio Negro: Adolfo Alsina.	G. Seijo 1454 (NY)	DQ478993	DQ479049		
Subtribe Brachycominae						
Brachycome basaltica F. Muell. var. *gracilis* Benth.	Australia: Oxley	P. G. Kodela 453 (MO)	x	DQ479050	x	
Subtribe Felicinae						
Amellus microglossus DC.	South Africa: Oranjemund: N. W. Cape; 700 m alt.	M. F. Thompson 159 (MO)	DQ478995	DQ479052	x	
Felicia aethiopica (Burm. f.) Bol. & W. Dod	South Africa: Cape Province.	E. Esterhysen 36321(MO)	DQ478997	DQ479054	x	
Felicia filifolia (DC.) Burtt-Davy ssp. *schaeferi* (Dinter) Grau	South Africa: Northern Cape Province	Bayer and Puttock SAF-96166 (CANB)			AF318911	Bayer and Cross 2003
Subtribe Grangeinae						
Egletes liebmannii Sch. Bip..	Mexico: Veracruz: Mun. Tempoal; 50 m alt.	M. Nee & B. F. Hansen 18367 (F)	DQ478978	DQ478963	x	
Plagiocheilus bogotensis (H.B.K.) Weddell	Ecuador: Chimborazo.	A. Freire Fierro 560 (QCA)	DQ479001	DQ479059	x	
Subtribe Hinterhuberinae						
***Chiliotrichum* group**						

table 5.1 continued

Chiliophyllum andinum Cabr.	Argentina: De San Juan: Angaco: Sierra de Pie de Palo; 1950-2050 m alt.	A. T. Hanziber et al. 23690 (F)	DQ479103	DQ479125	x
Chiliotrichopsis keideli Cabrera		P. Simon & Bonifancino, 520	DQ479105	DQ479127	x
Chiliotrichum rosmarinifolium Less.	Argentina: Neuquén	M. Bonifancino 336 (USA)	DQ479107	DQ479129	x
Diplostephium ericoides (Lam.) Cabrera	Ecuador; Cotopaxi-Napo border; 3800-3850 m alt.	J. L. Luteyn 13438 (LSU)	DQ479003	DQ479061	x
Lepidophyllum cupressiforme (Lam.) Cas.		M. Bonifancino 785	DQ479109	DQ479132	x
Nardophyllum chiliotrichoides A. Gray		M. Bonifancino 679	DQ479115	DQ479138	x
Oritrophium peruvianum (Lam.) Cuatr.	Ecuador: Loja; 3480-3600 m alt.	P. M. Jorgensen 2266 (MO)	DQ479117	DQ479141	x
Pteronia camphorata L. var. *camphorata*	S. Africa: Cape Province: Namaqualand, Khamiesberg; 1550 m alt.	K. Bremer 496 (MO)	DQ479118	DQ479142	x
***Hintehubera* group**					
Aztecaster matudae (Rzed.) Nesom	Mexico: Neuvo Leon; 2140 m alt.	Vankat 98 (TEX)	DQ479006	DQ479063	x
Floscaldasia hypsophila Cuatr.	Ecuador: Azuay: Cajas National Park; 4400 m alt.	P. Sklenar 2418 (USA)	DQ479009	DQ479065	x
Hinterhubera lanuginosa Cuatr. & Aristeg..	Venezuela: Merida: Distrito Rangel: Paramo de Mucuchie	S. E. Carpenter 893 (NY)	DQ479014	DQ479070	x
Laestadia muscicola (Sch. Bip.) Wedd.	Venezuela: Barinas: Paramo El Toro; 2480 m alt.	Briceño, B. et al. 2238 (MERC)	DQ479017	DQ479074	x
Westoniella triunguifolia Cuatr.	Costa Rica: San Jose: Cerro de la Muerte.	V. Karaman-Castro 239 (LSU)	DQ479030	DQ479086	x

table 5.1 continued

***Novenia* group**						
Novenia acaulis (Wedd. ex Benth) Freire & Hellwig	Peru: San Miguel: Cajamarca: Las Lagunas; 4000 m alt.	A. A. Sagástegui et al. 15719 (NY)	DQ479119	DQ479143	x	
***Olearia* group**						
Olearia covenyi Lander	Australia: New South Wales	A. M. Lyne 2218 (CANB)			AF456797	Bayer and Cross 2003
Olearia tomentosa (Wendl.) DC.	Australia: New South Wales	Makinson 1234 (CANB)			AF456796	Bayer and Cross 2003
unplaced						
Blakiella bartsiaefolia (Blake) Cuatr.	Venezuela: Merida: Rangel; 3000 m alt.	H. van der Werff 7707 (USA)	DQ479034	DQ479090	x	
Guynesomia scoparia (Phil.) Bonif. & G.Sancho	Chile: Elqui: Llanos de Huanta.	M. Bonifancino 820 (LP)	DQ479035	DQ479091	x	
Printzia polifolia (L.) Hutch.	South Africa: Western Cape Province	Bayer and Puttock SAF-96284 (CANB)			AF456799	Bayer and Cross 2003
Subtribe Lagenophorinae						
Keysseria maviensis (H. Mann) Cabrera	USA: Hawai: Maui: Puu Kukui trail. 5000 ft. alt.	S. Perlman 6875 (USA)	DQ479036	DQ479092	x	
Lagenophora pumila Cheeseman	New Zealand: South island: Boulder Lake Trail.	S. A. Norton 598 (NO)	DQ479037	DQ479094	x	
Subtribe Podocominae						
Sommerfeltia spinulosa Less.	Uruguay: Salto: Cerros del Boquerón de Ararunguá.	T. M. Pedersen 16281 (NY)	DQ479039	DQ479096	x	
Subtribe Symphyotrichinae						
Symphyotrichum novae-angliae (L.) Nesom	Commercially grown	Bayer AB-95003 (CANB)			AF151441	Bayer and Cross 2003

* Sequences from different species that were combined in the ITS/ETS/matK combined data set.

2003), and 814F (Panero and Crozier 2003) and 1848 R (Johnston and Soltis 1994) (fig. 5.1).

Amplifications were performed in 25 µl volumes containing: 1 µl of DNA template, 2.5 µl of 10x PCR buffer (Qiagen Inc., Valencia, CA), 2.5 µl of 5x Q-Solution (Qiagen Inc.), 0.5 µl of 25 mM $MgCl_2$, 0.5 µl of 10mM each dNTP, 0.2 µl of Taq DNA Polymerase (Qiagen Inc.), 15.3 µl of distilled water, and 1.25 µl of each 10 nM forward and reverse primer. The PCR protocol as outlined in Panero and Crozier (2003) consisted of initial preheating at 95°C for 5 min that was followed by 35 cycles of amplification as follows: denaturation at 95°C for 1 min; annealing at 48°C for 1 min with additional 2 s at each subsequent cycle; and extension at 72°C for 1 min. A final extension period of 10 min at 72°C finished the amplification. Sequencing was done using the 3914F-884R, and 814F-1848R primer pairs following protocol described in chapter 2.

Sequence Analyses and Phylogenetic Analyses. Sequences were edited and analyzed as outlined in chapter 2. Maximum parsimony and Bayesian analyses were performed on the matK data set alone, and on the combined ITS/ETS/matK sequence data. The three data sets were tested for their combinability using the partition homogeneity test (the incongruence length difference test of Farris et al. 1994) incorporated in Paup* 4.0b10 (Swoford 2000). The partition homogeneity test was implemented using TBR branch-swapping algorithm simple addition of sequences and saving 200 trees at each replicate.

Maximum parsimony (MP) was performed using Paup* 4.0b10. The heuristic searches used random stepwise taxon addition to obtain starting trees and the tree bisection-reconnection (TBR) branch swapping option, with steepest descent off. Nucleotide characters were unordered and equally weighted, and gaps were treated as missing data. Searches were

replicated 10,000 times. Relative clade support was calculated using bootstrap analyses with TBR branch swapping, 1000 bootstrap replicates, with 10 random addition replicates per bootstrap.

Bayesian inference of the two datasets was performed using MrBayes 3.1 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). Likelihood parameters for Bayesian analyses were calculated using MrModeltest 2.2 (Nylander 2004). GTR-I-Γ was chosen under AIC criteria (Buckley et al. 2002, Posada and Buckley 2004). Two independent runs were done, each starting from an independently generated random tree. Each Markov chain was run for $1.5x10^6$ generations (the matK dataset) and $2x10^6$ generations (combined dataset), and sampling was done each 200^{th}-cycle. Twenty five percent of the trees were discarded in burnin. The resulting 50% majority rule consensus tree was imported and observed in TreeView (Page 1996).

RESULTS

The matK dataset included 38 taxa. Because the sequences obtained from the GenBank were lacking the 5'trnK intron, the first 732 bp were deleted from the data matrix; they contained 28 (3%) potentially informative sites. Because of the missing data, an additional 96 bp at the 816F and 884R primers binding sites in the matK region were removed. The final aligned matrix equaled 1069 bp of which 851 (79.6%) characters were constant, 115 (10.8%) were parsimony uninformative, and 103 (9.6%) were parsimony informative. The matK/ETS/ITS combined data set included 28 taxa. The aligned matrix was 2014 bp long.

Because the overall topologies inferred from the matK data set robustly corresponded to the ITS and ETS topologies presented in chapter 4, the same terminology was used in labeling the main clades.

MP analyses of the matK data set yielded one island of 960 most parsimonious trees 319 steps long (CI=0.803, RI=0.817, RC=0.656). The strict consensus of these trees was congruent with the majority rule consensus tree inferred from the Bayesian analyses (fig. 5.2). Partition homogeneity test confirmed homogeneity of the matK and ITS/ETS datasets (p=0.722). The MP analyses of combined data set resulted in 7 trees 1921 steps long in two tree islands, and the strict consensus is shown in figure 5.3. The phylogram inferred from Bayesian analyses is presented in the figure 5.4. Topologies obtained from parsimony and Bayesian analyses were congruent with each other. The differences in the topologies were in the parts of the trees with low (PP<0.70, BS<60%) branch support.

Phylogenetic analyses of both datasets resolved two main clades (figs. 5.2-5.4), the basal group and the Southern Hemisphere grade (SH). The North American clade (NA) resolved in the matK dataset (fig. 5.2) was not presented in the combined dataset. Genera from the *Chiliotrichum* group, *Pteronia, Oritrophium, Novenia* and *Printzia*, and Felicinae were in the basal group. *Symphyotrichum novae-angliae* was the sole representative species of the NA clade. The NA clade and the SH grade identified in the molecular analyses of the ITS and ETS sequence data were unresolved in the matK-based phylogeny and constituted a terminal polytomy (fig. 5.2). The SH grade included taxa treated in the *Hinterhubera* group, Baccharidinae, Brachycominae, Lagenophorinae, Grangeinae and Podocominae (*sensu* Nesom 1994). An indel 6 bp long was synapomorphic for the SH grade and *S. novae-angliae*.

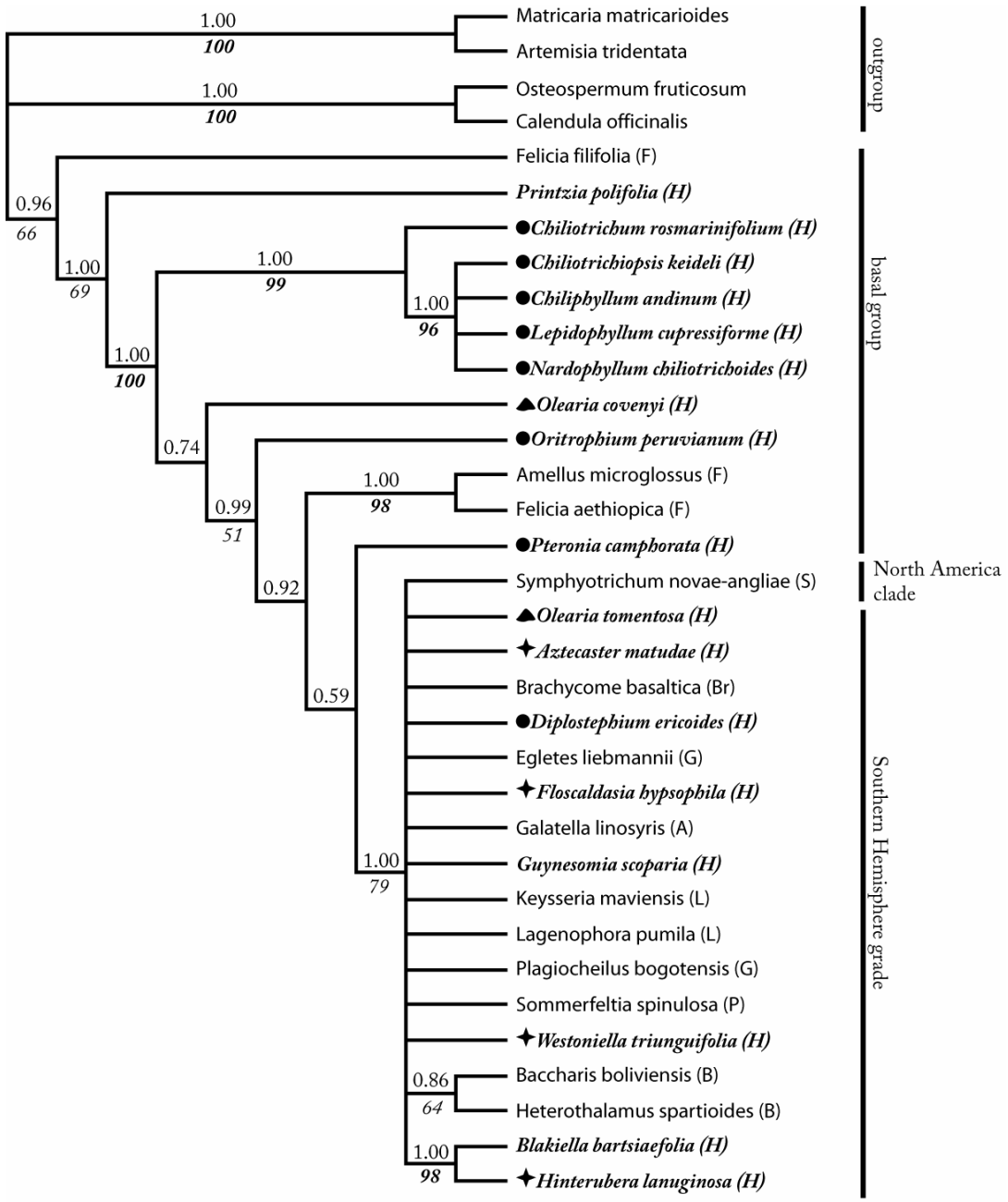

Figure 5.2. Phylogram inferred from Bayesian analyses of the matK sequence data and strict consensus of 960 most parsimonious trees. Posterior probabilities are decimal numbers above branches. Bootstrap support values (≤50% not shown) are below branches; values ≥90% are in bold. Subtribe abbreviations are as follow: A-Asterinae; B-Baccharidinae; Br-Brachycominae; F-Felicinae; G-Grangeinae; H- Hinterhuberinae; L-Lagenophorinae; P-Podocominae; S-Symphyotrichinae. Group abbreviations of Hinterhuberinae (*sensu* Nesom 1994) are as follow: ● - *Chiliotrichum*, ✦ – *Hinterhubera*, ▲ – *Olearia* group. Unlabeled Hinterhuberinae are currently not affiliated to any group.

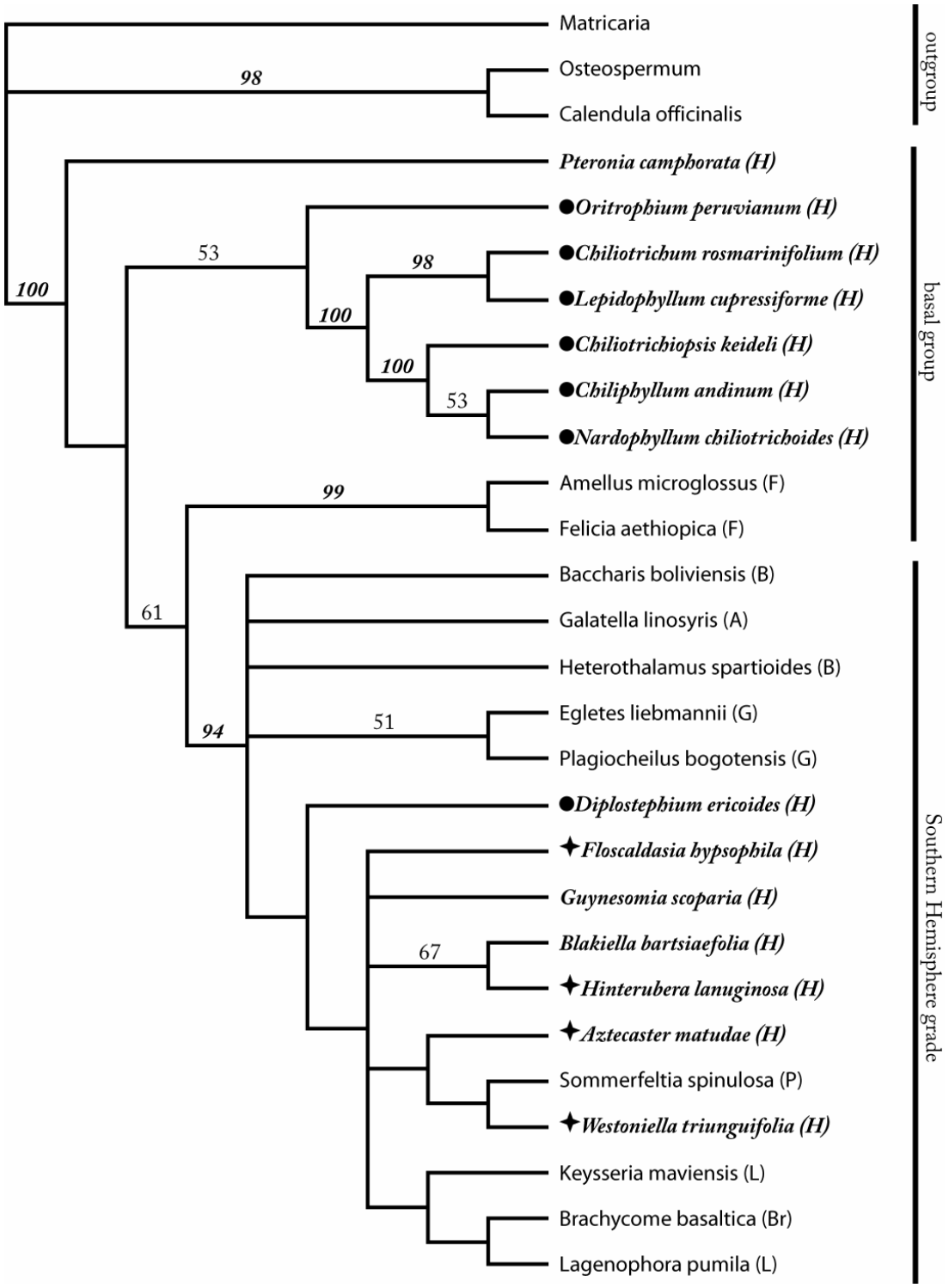

Figure 5.3. Strict consensus of 960 most parsimonious trees inferred from combined dataset. Bootstrap support values (values ≤50% not shown) are numbers above branches; values ≥90% are in bold. Subtribe and group abbreviations are given in figure 5.2.

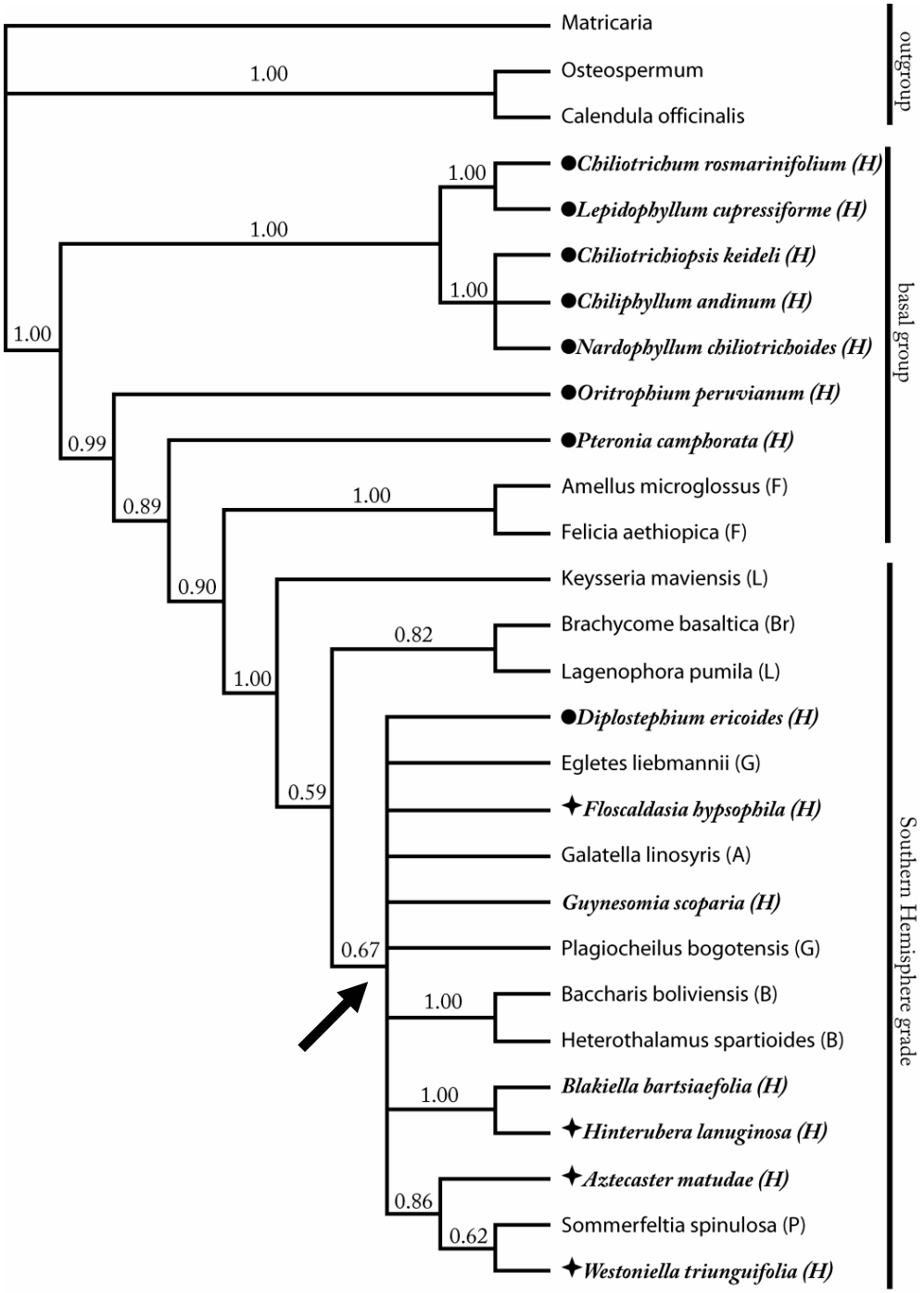

Figure 5.4. Phylogram inferred from Bayesian analyses of the combined sequence data. Posterior probabilities are decimal numbers above branches. Subtribe and group abbreviations are given in figure 5.2. The arrow delimits SA clade in the SH grade.

The SH grade was poorly resolved in matK-based analyses and partially resolved in the combined dataset (figs. 5.2, 5.4). This low resolution is due to the small pairwise divergence between the taxa from the SH grade where the maximum observed value was 4%, and the low phylogenetic signal in the matK dataset with only 10% parsimony informative characters. MrBayes inferred phylogeny of combined dataset recovered South American clade (SA) containing *Hinterhubera* group, Podocominae, Grangeinae and Baccharidinae (fig. 5.4). The Australasian and Hawaiian genera, *Brachycome*, *Keysseria* and *Lagenophora*, similarly to the ETS and ITS based topologies (figs. 4.1, 4.3), were basal to this SA clade. This better resolution of the SH grade in the combined dataset was influenced by the ETS/ITS data which contributed 77% of the phylogenetic signal compared to 23% by matK.

DISCUSSION

Phylogenetic analyses based on matK data confirmed findings suggested by the nrDNA sequence data that Hinterhuberinae (*sensu* Nesom 1994) is not monophyletic. The near basal position of the *Chiliotrichum* group in the tribe Astereae in the matK phylogeny corroborated the ETS/ITS results that the group has little affiliation with the *Hinterhubera* group and should in fact be excluded from the subtribe. The position of the Australasian *Celmisia* was not investigated with the cpDNA data. Based on the similar topologies recovered in the analyses of these two different and unlinked datasets, nrDNA and matK, it is likely that matK data will resolve *Celmisia* group in a lineage sister to the *Chiliotrichum* group.

The relationship between taxa in the SH grade was poorly resolved. The sister relationship of *Hinterhubera* and *Blakiella* in both data sets recovered with modest to high support (PP=1.00, BS=98%, figs. 5.2, 5.4, BS=67%, fig. 5.3) is of great interest since

Blakiella's affinities have been questionable (Cuatrecasas 1986, Nesom 1994, Nesom and Robinson 2006; see chapter 2). Similarly, the *Aztecaster-Westoniella* clade was recovered with moderate support in the combined dataset (PP=0.86, fig. 5.4). Because of the unavailability of matK sequence data for a number of taxa the relationships between *Archibaccharis* and *Plagiocheilus*, *Laestadia, Hinterhubera* and *Blakiella*, and *Westoniella* and *Laennecia* could not be tested.

Recent molecular analyses based on three cpDNA data, the trnL/F spacer, the trnL intron and the matK coding region (Bayer and Cross 2002) suggested that the southern African genus *Printzia* Cass. belongs to the tribe Astereae. Having been considered within tribes Inuleae, Astereae and Mutisieae by various authors, the relationship of this genus has been controversial as reviewed by Bayer and Cross (2002). Because of its long-tailed anthers it was placed in Inuleae, while morphology of leaves, capitula, cypselae and pappus with the exception of pollen and obtuse style branches are similar to South American Astereae, in particular *Olearia* (Hinterhuberinae), as discussed in Bayer and Cross (2002). Based on the basal position of *Printzia* in the tribe Astereae recovered in the molecular analyses of Bayer and Cross (2002) the genus was included in Hinterhuberinae by Nesom and Robinson (2006) with no further explanation. The analyses of the matK data presented in this study supported the basal position of *Printzia* within Astereae (fig. 5.2). Although data did support *Printzia'*s basal position to the *Chiliotrichum* group, they did not justify its transfer to Hinterhuberinae. That *Printzia* does not belong to the Hinterhuberinae was also confirmed by the analysis of the trnL intron/ trnL-F spacers dataset (data not shown). A small data set was created that included accessions obtained from the GenBank. It included one accession of *Printzia*, three species from two genera in the *Chilitiorichum* group, one species of *Symphyotrichum* (Bayer

and Cross 2002), one accession of *Townsendia*, and two species of *Nannoglottis* Maxim (Liu et al. 2002). *Nannoglottis,* a genus from the Qinghai-Tibet Plateau, was a first diverging lineage in the tribe Astereae (Liu et al. 2002). Parsimony, likelihood and MrBayes analyses of the trnL-trnF dataset with Anthemideae designated as outgroup, resolved *Printzia* in a sister clade with *Nannoglottis*. Such a relationship in addition with the molecular analyses of Liu et al. (2002) suggests the most basal position of *Printzia* in the tribe Astereae. Further sampling of Asian taxa is of great interest to help understand the relationships of *Printzia* and *Nannoglottis* to other Astereae.

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CHAPTER 6 SUMMARY AND CONCLUSIONS

Molecular analyses of Hinterhuberinae based on the ETS, ITS and matK sequence failed the test of monophyly. The *Chiliotrichum* (excluding *Diplostephium)*, *Celmisia* and *Novenia* groups, are part of the basal group, while *Madagaster*, *Olearia* and *Hinterhubera* groups, including *Diplostephium, Guynesomia* and *Blakiella* are in the Southern Hemisphere grade with Lagenophorinae, Asterinae, Podocominae, Baccharidinae, and Brachycominae.

HINTERHUBERA AND RELATED GENERA

Hinterhubera group, Lagenophorinae, Podocominae, Baccharidinae and three other genera from the subtribe Hinterhuberinae, *Blakiella*, *Diplostephium* and *Guynesomia* group in a South American clade that is nested within the Southern Hemisphere grade. They are distributed in South and Central America and Mexico. The subtribe Brachycominae, Asterinae, Lagenophorinae, Podocominae, then *Remya*, *Olearia* in part and *Achnophora* from Hinterhuberinae, that are distributed in Australia, New Zealand, New Guinea, Hawaii, Africa or Eurasia are in the Austral grade that is sister to the South American clade. Such phylogenies suggest a closer relationship among taxa from similar geographic areas rather than according to their existing subtribal assignments.

Monophyly of the genera within the group is confirmed in all analyses except for the genus *Laestadia*. The relationships of *L. pinnifolia* were not resolved in the ETS-based phylogenies. *Laestadia* is sister to *Hinterhubera* and *Blakiella*, hence, the presence of similar beaked and epappose cypselae in Grangeinae and *Laestadia* is due to the convergent evolution. *Westoniella* is in a clade with *Laennecia* (Podocominae). Interspecific relationships of *Parastrephia* are not resolved. Four of the five species are grouped in two sister clades, each containing accessions of *P. lucida* and *P. quadrangularis*, and either *P. lepidophylla* or

P. phylicaeformis. Presence of multiple terminals of the same species on the topology is a result of either lineage sorting or interspecific hybridization. *Floscaldasia* is unresolved to the other genera from the *Hinterhubera* group. Phylogenetic placement of *Diplostephium* and *Guynesomia scoparia* in the *Hinterhubera* group suggests that evolutionary tendency within Hinterhuberinae is the transition from radiate capitula with bisexual disk florets towards taxa with discoid and disciform capitula and staminate disk florets, and finally dioecy.

Madagaster has isolated position within the tribe Astereae and is basal in the Southern Hemisphere grade, questioning its placement in Hinterhuberinae. Hawaiian *Remya* is with Australasian genera of Podocominae, Lagenophorinae and Brachycominae in the polytomy basal to the *Hinterhubera* group. The same polytomy also includes *Olearia* in part, and *Achnophora*. The later is not related to *Olearia* or any other genus from Hinterhuberinae.

Dioecy in the tribe Astereae is present in Baccharidinae and *Aztecaster*. Molecular data support its independent evolution in these two groups. The pistillate capitula in *Aztecaster* may have evolved from discoid or possibly radiate capitula by complete reduction of ligules in marginal pistillate corollas and loss of staminate disk florets. Another possibility is that the pistillate corollas evolved from bisexual, tubular disk florets.

Baccharidinae, including the genus *Plagiocheilus* (Grangeinae), are monophyletic. Sister relationship between *Archibaccharis* and *Plagiocheilus* in the ETS/ITS based analyses is not supported by morphological characters and needs further research.

Felicinae are resolved basal to the remaining Astereae, or basal to the Southern Hemisphere grade, in which case the *Chiliotrichum*-*Celmisia* clade is the most basal in the tribe Astereae.

***CHILIOTRICHUM*, *NOVENIA* AND RELATED GENERA**

The monophyly of the South American *Chiliotrichum* group is not supported in the ITS and ETS based phylogenies. *Diplostephium* is placed within the Southern Hemisphere grade, and only distantly related to other genera from the *Chiliotrichum* group. The two herbaceous perennial genera, *Oritrophium* and *Novenia* form a clade that is apart from the *Chiliotrichum* clade. The two genera group with the shrubby southern African *Pteronia*, and all three are basal to the Southern Hemisphere grade. Sister relationship with *Pteronia* received low support. Examination of *Oritrophium* and *Pteronia* specimens revealed similarity in the style branches shape, a feature that needs further investigation.

The remaining *Chiliotrichum* group forms a monophyletic group that is sister to the *Celmisia* clade. *Chiliotrichum* and *Celmisia* clades include genera that are characterized by discoid or radiate capitula, paleate to partially paleate or epaleate receptacle, and hermaphroditic disk florets (they are functionally staminate in *Lepidophyllum*). The genus *Chiliophyllum* is polyphyletic, *C. fuegianum* is sister to *Chiliotrichum* and *Chiliophyllum andinum* is sister to *Nardophyllum*. This molecular data suggest that the color of ray florets and morphology of pappus bristles, characters traditionally used in delimiting this genus, are homoplastic for the genus *Chiliophyllum*. *Llerasia* is either basal in the *Chiliotrichum* clade or is sister to *Nardophyllum*. Partially paleate to epaleate receptacle and discoid capitula support its relationship to *Nardophyllum*. Difference in the habit and cypselae pubescence warrants further study.

Geographical distribution of the genera from the *Chiliotrichum* and *Novenia* clades, with *Oritrophium, Novenia* and *Llerasia* being distributed in the northern part of South America and *Chiliotrichum* clade in the southern half of the South America is probably the

result of barriers present in Pleistocene glacial time at and around the extended lake system of Titicaca, and extensive glaciations of the Cordillera Real east of La Paz.

High level of polyploidy in *Celmisia* and *Chiliotrichum* clades (hexaploids to dodecaploids) is probably a result of an ancient polyploidy.

MORPHOLOGICAL MAPPING AND BIOGEOGRAPHY

Woody habit is resolved ancestral in the tribe Astereae. Herbaceous habit in the basal group is restricted to *Oritrophium, Novenia*, some *Celmisia* and Felicinae, but it is prevalent in the North American clade. Woody habit in some of the genera from the North American clade may be a result of a secondary reversion from herbaceous to woody form. The Southern Hemisphere grade contains woody and herbaceous genera and no coherent tendency in the evolution of the forms is observed within the grade.

Receptacular paleae are characteristic of the *Chiliotrichum* group. In *Amellus* (Felicinae), *Chiliotrichum* group, *Achnophora, Diplostephium* (Hinterhuberinae), *Heterothalamus* (Baccharidinae) and in two taxa from the North American clade paleae evolved independently.

The plesiomorphic type of disk florets is fully fertile, bisexual floret as found in *Chiliotrichum* and *Celmisia* clades. Staminate disk florets evolved independently in several lineages: Felicinae, *Novenia-Oritrophium* clade, Southern Hemisphere grade and North American clade. In the Southern Hemisphere grade, disk florets are functionally staminate in Podocominae (*Tetramolopium*, *Minuria*), Lagenophorinae (*Myriactis*) and Hinterhuberinae (*Hinterhubera* group, *Achnophora* and *Remya*)

Basal group is characterized by radiate capitula, with few genera having reduced ligule or discoid capitula (*Novenia, Lepidophyllum* and *Llerasia*). The Southern Hemisphere grade is

more variable in ligule morphology. The *Hinterhubera* group, in general includes genera with reduced ligule or the pistillate corollas are tubular with long asymmetric lobes. *Diplostephium* and *Guynesomia* have radiate capitula and *Aztecaster* tubular actinomorphic corollas. It is possible that the reduction of ligule is adaptation to wind pollination or in the case of cushiony plants with clustered capitula, the presence of ligule is redundant.

Molecular data support redefinition of the subtribe Hinterhuberinae. The new redefined Hinterhuberinae include *Hinterhubera* group, *Guynesomia, Diplostephium*, and *Blakiella*. *Remya, Achnophora* and *Olearia* in part however, although part of the Southern Hemisphere grade, are not related to the *Hinterhubera* group and their placement within Hinterhuberinae is questionable.

Chiliotrichum and *Celmisia* clades are proposed to be excluded from the subtribe in a new group that is, together with Felicinae, closest to the ancestral stock of Astereae. Molecular analyses suggest inclusion of *Novenia-Oritrophium* clade and the genus *Pteronia* within this new group, but this is not be supported by morphological characters.

Current generic delimitation and classification of the Southern Hemisphere subtribes is questioned. Podocominae, Lagenophorinae, Grangeinae and Asterinae are polyphyletic and further sampling of taxa from these subtribes is needed.

South America, southern Africa and New Zealand are proposed as the three possible centers of origin for the tribe Astereae. Dispersion between continents was possible over the stepping-stones and long distance dispersal in the Eocene or Oligocene.

Chiliotrichum and *Celmisia* clades originated either in New Zealand, South America or southern Africa. Dispersal between New Zealand and South America was probably happening through Antarctica that functioned as a bridge between these continents.

Hinterhubera group evolved recently from the South American lower altitude autochthonous elements with the uplift of the Andes. It is likely that the genera spread from the southern and central Andes during the ice age when the páramo zone became wider covering lower elevations and thus allowing species to spread between different “islands” of mountain peaks.

VITA

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